

Epigenetics, resilience and brain stimulation

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EPIGENETICS, RESILIENCE, AND BRAIN STIMULATION

ADVANCES IN THE MECHANISTIC AND
THERAPEUTIC UTILITY IN PATIENTS WITH
AFFECTIVE DISORDERS

by

Nagy A. Youssef, MD

CH₃

Artwork by Anna Youssef

**EPIGENETICS, RESILIENCE, AND BRAIN STIMULATION: ADVANCES IN
THE MECHANISTIC AND THERAPEUTIC UTILITY IN PATIENTS WITH
AFFECTIVE (PTSD AND MOOD) DISORDERS**

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Chapter 1 General Introduction

INTRODUCTION

Psychological adaptation reflects functional, cognitive and/or behavioral adjustment to contextual (environmental) changes that help the individual and comprises a key factor in psychological well-being. The mechanisms underlying adaptability to environmental challenges as well as the onset and course of mental disorders comprise biopsychosocial and existential factors. Based on advances in methodologies and conceptual ideas on how nature and nurture may interact, there is now ample opportunity to identify such factors and to advance our understanding. At the molecular biological level, epigenetic mechanisms may have an important role in the response to rapid environmental change and adaptation. For example, people who are resilient to disease development may have epigenetic makeup that fosters the adaptive processes to the environmental exposures during life. One of the newly evolving research and clinical tools that can be used to both modify neuronal function, adaptability, and neuroplasticity and to investigate underlying (biological) processes is brain stimulation (Bolwig & Madsen, 2007; Joshi et al., 2016; Schloesser et al., 2015). Stimulation of the brain reflects exogenously induced or administered interventions and can thus be seen as an environmental exposure.

This thesis focuses and converges on two promising and rapidly evolving areas of neuroscience and psychiatry. These areas are epigenetics and brain stimulation. Research in both of these areas not only has the potential to transform our understanding of the onset and course of mental illness in high risk populations, but also in understanding resilience against mental illnesses in those who can “bounce back” from adversities with no development of mental illness (despite exposure to a similar environment).

Although the two main sections of this thesis, namely brain stimulation on one side and resilience and epigenetics on the other side, are not always related, some early studies in the literature have examined the relationship and interactions between both. For instance, there is evidence to suggest from Professor Rutten’s laboratory that brain stimulation interventions may lead to epigenetic changes (de Jong JO et al., 2014). My hope is that this thesis will bring us a step forward to answering some of these questions in the two fields and spur interest in how both of these 2 fields can interact together and benefit from each other.

As for future directions after this thesis, I would like to investigate how brain stimulation interventions might improve resilience. An ECT study from our group, where I was the principal investigator, supports that patients who have undergone ECT for treatment-resistant depression also experienced improved resilience (publication pending). These relations might be mediated by epigenetics. I believe this to be important area to be explored further in the future as a follow up after my PhD.

On the other hand, epigenetics’ signature may provide markers of treatment response for ECT or other brain stimulation interventions. The clinical implications of this are great. For instance, knowing beforehand through epigenetic testing who will respond to, or who will not respond to an acute course of ECT, can save a lot of suffering, cost, and time. This can individualize treatment to the right individual.

Of course, more studies in these areas are needed in the future, and thus one of the goals of this thesis is to stimulate more studies about the interactions of the two areas. It is hoped that it spurs further studies in the field to contribute to our understanding of these two interesting fields and their relationships. Although this thesis will address these two areas separately, given my interest in both, the work in this thesis will act as preparation and a sounding board for me for the next steps after my PhD, which is to examine and perform studies that involve interactions between both areas to further understand the above relationships.

The first section of this thesis focuses on epigenetics as one possible molecular mechanism underlying risk and resilience to psychological trauma in relation to subsequent development of affective disorders such as PTSD and depression.

Several studies in the literature have described the relationship between **resilience** and **epigenetics**, where epigenetic alterations might cause certain individuals to be more resilient to the effects of trauma and depression than others. Resilience does not only have a psychological contribution but also a biological contribution, which probably has an epigenetic basis.

The second section of this thesis will focus on electroconvulsive therapy (ECT). Of the brain stimulation interventions, ECT is considered to be not only the oldest (with much improved modern techniques for application), but also the most effective treatment known to man for several psychiatric disorders, especially mood disorders (Greenberg & Kellner, 2005; Lin, Huang, & Chen, 2018). Thus, ECT is considered the prototype of brain stimulation techniques and will be the focus of this thesis.

Moreover, ECT has the most research support among neuromodulation devices. In this thesis, I will also shed some light on cutting-edge research developed to mitigate (or avoid) cognitive side effects of ECT and how modern modification has markedly improved the safety and tolerability of ECT.

General introduction on epigenetics and its role in mental disorders:

The field of epigenetics may offer useful insights into differential susceptibility of risk to develop psychopathology as well as resilience. According to Goldberg et al., epigenetics is the study of functionally stable and (ideally) heritable changes in gene expression or cellular phenotype that occurs without changes in base pairing (Goldberg, Allis, & Bernstein, 2007). The National Institutes of Health in the United States provides a broader definition, wherein their 2009 epigenomics initiative stated that “epigenetics refers to both heritable changes in gene activity and expression (in the progeny of cells or of individuals) and also stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable” (National Advisory Mental Health Council Workgroup on Genomics, 2018).

Thus, epigenetics involves functional changes to the gene without sequence changes. The best-studied epigenetic mechanism so far is DNA methylation. DNA methylation involves the

attachment of methyl groups to the DNA molecule. When methyl groups are attached to the promoter region, they typically act to repress gene transcription.

Certain DNA methylation alterations have been associated with several mental phenotypes. For instance, we found an association between hypermethylation of either Brain-derived neurotrophic factor (BDNF) promoter or tropomyosin receptor kinase B (TrkB) and suicide (Lockwood, Su, & Youssef, 2015).

Interactions between the environment and biological background of an individual during the onset and course of mental disorders may be better understood in the lens of epigenetics. This is because epigenetics may be an important link that explains how the environment (trauma or stressor) interfaces with the biological machinery. The exposure to psychological trauma has been shown to increase risk of the onset of PTSD and depression (Hovens, Giltay, Spinhoven, van Hemert, & Penninx, 2015; Hovens et al., 2012; Youssef et al., 2017; Youssef et al., 2013). Also, chronic severe stress has been observed to exacerbate or induce an episode of depression. Childhood trauma has even been associated with the development of physical illnesses such as cardiometabolic disease in adulthood, and evidence suggests the involvement of an epigenetic mechanism (Hao, Youssef, Davis, & Su, 2018).

An important question that frequently arises is: Why do some people develop mental disorders after experiencing a traumatic event, while others do not? Possible explanations could be an underlying genetic or epigenetic risk in those who are more prone to develop PTSD or depression. Similarly, those who are resilient to disease development may have some epigenetic makeup that facilitates the adaptive processes.

In this example, PTSD has long been established to be due to exposure to trauma (trauma is even the cardinal criterion for PTSD; without it PTSD cannot be diagnosed). Thus, it has been assumed that only environmental factors, namely trauma, contribute to the development of PTSD. However, emerging data show that: Although trauma is necessary, it is not sufficient for PTSD development. A range of studies has now estimated that PTSD shows considerable heritability component that is between 30% and 70% in twin studies (Sartor et al., 2012; Stein, Jang, Taylor, Vernon, & Livesley, 2002; True et al., 1993; Xian et al., 2000). This indicates the role of heritable factors related to either DNA structure or function. Then again, many decades of genetic studies of both candidate gene and genome-wide association studies (GWAS) have provided many interesting and promising findings, yet so far no robust structural genetic variants for PTSD have been identified (Logue et al., 2015). This risk is unlikely to be fully explained by only structural genetics (Ratanatharathorn et al., 2017). Thus, the field of epigenetics could offer great insights into differential susceptibility of risk to develop psychopathology.

Epigenetics, gene expression, and cell type specificity:

Although all of our cells contain the same DNA, the cell types and functions vary greatly, resulting in different organs. This is due to both qualitative and quantitative differences in their gene expression.

The transfer of genetic information from the DNA to the messenger RNA (mRNA), (usually resulting in the subsequent protein formation or modification) is done through transcription then translation into proteins.

Epigenetic mechanisms, including DNA methylation, among others, influence gene expression mainly at the level of transcription. However, translation may also be regulated epigenetically.

Epigenetic response to environmental change and epigenetic mediation of neuronal plasticity:

Epigenetic mechanisms may have an important role in the response to rapid environmental change and adaptation (Schrey et al., 2016). This may involve behavioral or physiological adaptation. However, certain environmental stressors and trauma might lead to dysfunctional responses that lead to symptoms and disorders in susceptible individuals, while not in others.

The epigenetic alteration in the response to rapid environmental changes also requires an interface between epigenetics and neuronal plasticity. The interaction between epigenetics and plasticity can be in the form of either functional (physiological) or structural changes in the neurons in response to internal or external stimuli and stressors. If the adaption is dysfunctional, or the system is overwhelmed and unable to adapt altogether, this can lead to disease development. Likewise, in other individuals, the physiological changes lead to successful functional adaptation (and possibly structural), and thus amount to rapid recovery from trauma or resilience against trauma or stressors in the face of the same type and intensity of the trauma/stressor.

General introduction to brain stimulation and approaches for stimulation:

As for the field of brain stimulation, it commonly involves either electric or magnetic stimulation of particular brain circuit(s) or region(s) to modulate (stimulation, inhibition, etc.) neuronal activity in specific circuit(s) or region(s) of the brain. Such brain stimulation interventions can in principle be used to modulate neuronal activity either for therapeutic reasons as commonly used clinically (as well as for research purposes to develop therapeutic interventions), or as a probe in order to identify certain conditions or states (as a biomarker). The use of brain stimulation modalities as probes/biomarkers is now mostly confined to research and is not yet in clinical use. Brain stimulation modalities involve transcranial, noninvasive stimulation, but can also be intracranial as in the case of deep brain stimulation (DBS). ECT is the

oldest and most effective method of noninvasive brain stimulation that is commonly used in clinical practice. ECT is a prototype of brain stimulation and will be the focus of the brain stimulation section of this thesis.

Brain stimulation comprises the administration of a direct, specific environmental stimulus that can modify neuronal functioning, neuroplasticity, and possibly epigenetics. Indeed, not only can electric or magnetic brain stimulation modify neuronal functioning for fostering treatment and recovery, but it can also be considered as a tool in boosting resilience (Bolwig & Madsen, 2007; Joshi et al., 2016; Schloesser et al., 2015).

Brain stimulation can provide a focused environmental stimulus to certain regions of the brain, and some have even considered brain stimulatory approaches for the restoration of healthy states as well as for the promotion of healing and recovery (Veena et al., 2009).

Environmental enrichment through offering enhanced positive stimulation to an individual has been suggested to improve brain function. Environmental enrichment has also been connected to structural neuronal and synaptic formation (Fan, Liu, Weinstein, Fike, & Liu, 2007; Veena et al., 2009), prevention (and even reversal) of neuronal loss in the hippocampus (after chronic stress) by inducing adult hippocampal regeneration of neurons (Veena et al., 2009), and possibly hippocampal neurogenesis (Cao et al., 2018; Joshi et al., 2016; Schloesser et al., 2015; Tendolkar et al., 2013). This has also been observed in studies using other brain stimulation modalities such as vagal nerve stimulation, suggesting a “modulatory effect on hippocampal plasticity” as mentioned by the Perini and colleagues’ study (Perini et al., 2017).

What main questions have arisen in the fields of epigenetics and brain stimulation?

There are/remain a lot of unanswered questions, both in the field of epigenetics and brain stimulation. Some of these important questions are as follows:

- Is there evidence that depression is associated with epigenetic alterations? Similarly, is there evidence that epigenetic alterations are associated with suicide or suicidality? Are there transgenerational effects of trauma and/or PTSD, or does trauma only affect the person exposed to it?

Answers to these questions and many more in epigenetics, as well as replication of existing studies in a larger dataset and in diverse populations, will be improved by data sharing between many centers. Sharing data will generate a substantial sample size and meaningful strong significances in genome-wide studies (Ratanatharathorn et al., 2017), as indicated also by the National Advisory Mental Health Council Workgroup on Genomics, 2018 (NAMHC) (National Advisory Mental Health Council Workgroup on Genomics, 2018). Thus, it is key to ask:

- Is it feasible to assemble an epigenetics consortium to share and pool data for generating a large sample size (to increase the power of detection) in epigenetic studies?

Other important questions on the clinical side are:

- What is the meaning of a certain traumatic event (*e.g.* combat trauma) to the specific individual? Does the moral code and/or their spiritual or religious beliefs color the clinical presentation in that it has an effect on development or lack of development of psychopathology?
- What is the relationship between moral injury and severity of PTSD symptoms in U.S. military veterans? Are there mediating and moderating effects of religiosity on the relationship between moral injury and PTSD severity?

Moral injury has been defined by Litz and colleagues as “perpetrating, failing to prevent, bearing witness to, or learning about acts that transgress deeply held moral beliefs.” Moral injury, a construct that is different from PTSD, has been described after traumatic experiences during wartime (Litz et al., 2009; Nash & Litz, 2013; Shay, 1994) and can co-occur with PTSD.

Moral injury results from experiences that violate deeply held moral beliefs and values, which then arouse shame, guilt, and existential questioning. Moral injury symptoms include both psychological and spiritual symptoms (loss of meaning and hope, spiritual struggles, loss of faith) (Koenig et al., 2017). Treatments targeting moral injury (both spiritual and secular) have been shown to reduce PTSD symptoms (Gray et al., 2012; Ogden et al., 2011).

Other related important questions in this clinical area include:

- Is there a dose-response relationship between trauma and depressive symptoms?
- Is early childhood trauma effect different or similar across difference races? For instance, does early childhood trauma affect European Americans (EA) and African Americans (AA) in a similar fashion?
- Does resilience mitigate the effect of early childhood trauma on depressive symptoms?
- What is the effect of resilience on suicidality? More specifically, would higher resilience decrease the risk of suicide, thus have a protective effect against suicidality?

Some overarching and important questions in the field of brain stimulation are as follows:

- What are the best strategies to avoid relapse and recurrence of depressive symptoms after an acute ECT course?

- Does continuation ECT plus medications have superior impact on quality of life after remission of a depressive episode with an acute course of ECT compared to continuation medications alone?
- What is the current state of knowledge in the field of ECT regarding indications, patient selection, choice of electrode placement, and stimulus parameters?
- What are the future directions of research in the field of ECT including biomarkers and new techniques for delivering ECT?
- Is there preliminary human data that ECT can also be effective for PTSD, especially treatment-resistant PTSD? What is the feasibility (especially for the ability to consistently induce seizures) and safety of using a low current amplitude 0.5-0.6A (Low Pulse Amplitude Seizure Therapy or LAP-ST) for treatment of depressive episodes? Is there preliminary evidence that LAP-ST has fewer cognitive adverse effects compared to standard ECT while retaining efficacy?

Overview of thesis

This thesis attempts to answer most of these questions both in the fields of epigenetics as well as brain stimulation. It addresses these fields into the context of two main disease classifications (both with significant genetic-environmental (GxE) interaction) namely mood disorders and PTSD. Thus, the research compiled in this thesis can be divided in two main sections. The first section focuses on ‘Epigenetics and Resilience,’ and the second section focuses on ‘Brain Stimulation’ with ECT as the prototype for brain stimulation treatments for the purpose of this thesis. The thesis will also introduce a novel form of ECT, namely Low Amplitude Seizure Therapy (LAP-ST).

The **first section** focuses on epigenetics and resilience and starts with **Chapter 2**, which defines epigenetics, and critically reviews the available studies on the association between epigenetics and depression.

In order to examine the available literature on the effects of individual trauma exposure and PTSD on the offspring, and if there is a probability of transgenerational transmission via epigenetic alterations, **Chapter 3** critically reviews the human literature that discusses and studies the possible role of epigenetic methylation transmission from one generation to the next. The chapter also concludes with a discussion of the challenges that the field of epigenetics faces and provides guidelines for improving the quality of future epigenetic research.

In order to examine the feasibility to assemble an epigenetics consortium to share and pool data for generating a large sample size, **Chapter 4** describes our efforts in assembling a consortium to develop a common pipeline for Epigenome-Wide Association of PTSD collected

from many centers to increase the number of participants and statistical power needed for epigenetic research. These data from different cohorts/centers will have a Common Multi-Site Analysis Pipeline.

Since epigenetics may also play a role in resilience, the following chapters (Chapters 5, 6, and 7) switch gears from the molecular level to the clinical level by presenting studies of resilience to mental problems. Given that spirituality has been described as one of the domains for the Conner Davidson Resilience Scale (CD-RISC) and that moral values and meaning of life (Snijders et al., 2018) have been suggested to influence resilience, we examine spirituality and moral injury in veterans with PTSD.

In order to examine the effect of resilience on suicidality and if higher resilience decreases the risk of suicide, **Chapter 5** presents data from our 3-year longitudinal study of 178 veterans that examines this relationship.

In order to examine whether there is a dose-response relationship between trauma and depressive symptoms, whether early trauma affects European Americans and African Americans in a similar fashion, and whether resilience mitigates the effect of trauma on depression, **Chapter 6** presents our study of 413 participants to answer these questions.

Chapter 7 describes our cross-sectional study of 120 U.S. combat veterans in an effort to answer inquiries related to 1) the meaning of combat trauma to a specific individual based on their moral code and/or spiritual or religious beliefs, 2) the relationship between moral injury and severity of PTSD symptoms in U.S. military veterans, and 3) any mediating and moderating effects of religiosity on the relationship between moral injury and PTSD severity.

Section two of the thesis focuses on ECT. Treatment-resistant depression (TRD) is one of the most common indications for ECT. Thus, the section starts with **Chapter 8**, which presents a critical systematic review of the literature to examine the best way to prevent relapse in TRD after remission of an episode, and whether ECT alone, pharmacology alone, or combined ECT and pharmacology would be the best way to prevent relapse after an acute course of ECT for TRD.

In those who had full remission of depressive symptoms after an acute course of ECT, the question then becomes: Does continuation of ECT plus medications have a superior impact on quality of life compared to continuation of medications alone? Thus, **Chapter 9** presents our randomized multicenter clinical trial on the effects of continuation of ECT plus medications versus continuation of medication alone on improving the quality in a total sample of 120 patients to address this question.

In order to briefly overview the literature on the current knowledge of ECT and the future direction of ECT, **Chapter 10** discusses the achievements so far in ECT, including current knowledge on indication, patient selection, choice of electrode placement and stimulus parameters, augmentation with pharmacotherapy, and the use of continuation/maintenance treatment. Then, it discusses cutting-edge research in the field including research into

biomarkers and neuroplasticity related to ECT response, as well as new, investigational methods of ECT delivery. Then, it charts the sea of future research of “where we are headed. “

In order to examine any available evidence that ECT might be of help in PTSD (especially for treatment-resistant cases) and if this warrants a definitive randomized clinical trial, **Chapter 11** critically reviews the literature of the potential use of ECT for PTSD by reviewing the emerging literature and providing directions for future research.

Although ECT is the most effective treatment for TRD, cognitive (including memory) side effects can both be burdensome to some patients who were treated with ECT, and may discourage others who desperately need this life-saving intervention from receiving it. Thus, in order to find ways to avoid (or minimize) the cognitive side effects, we examine a more focal form of stimulation: Low Pulse Amplitude Seizure Therapy (LAP-ST) for treatment of depressive episodes. In this clinical trial, we specifically examine the feasibility and any preliminary evidence that it has less cognitive adverse effects compared to standard ECT. Thus, **Chapter 12** presents this investigational technique and the first open-label proof of concept (POC) clinical trial using LAP-ST to examine this technique and answer these questions. **Chapter 13** is a “Discussion” of the concepts presented in this thesis, including the summary, limitations, and recommended future research directions.

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Chapter 2 The Role of Epigenetics in Depression and Suicide: A Platform for Gene-Environment Interactions

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Abstract

Epigenetics involves functional modifications of genes that are affected by environmental factors. The aim of this paper is to explore if an association exists between epigenetics and depression and/or suicide. Medline/PubMed searches were performed using both Medical Subject Heading (MeSH) and Non-MeSH terms. Based on pre-specified terms and inclusion criteria, sixteen studies met inclusion criteria by 2 independent reviewers. Epigenetic changes seem to be important in both depression and suicide. All of the studies reviewed herein found significant epigenetic changes associated with depression and suicide except for two. Several studies showed that hypermethylation of BDNF is involved in suicide. TrkB hypermethylation was also shown to be associated with suicide by several studies, specifically in Brodmann's Areas (BA) 8 and 9. Future research is needed in a larger sample to further characterize these changes.

Keywords

Epigenetics, Depression, Suicide, Suicidality, Genes, Methylation of Genes, Gene-Environment Interactions

1. INTRODUCTION

Epigenetics involves functional modifications of genes that are affected by environmental factors. Goldberg et al (2007) defines epigenetics in a broad sense as a “bridge between genotype and phenotype.” They also define it more specifically as “the study of any potentially stable and, ideally, heritable changes in gene expression or cellular phenotype that occurs without changes in Watson-Crick base-pairing of DNA” (Goldberg, Allis, & Bernstein, 2007). Epigenetics involves functional changes to the genome that may result in it being “turned off” or “turned on,” and does not involve a change in the nucleotide sequence or structure. “Epigenetic markers on DNA can be influenced by environmental forces, such as smoking, diet, and pollutants” (Yan, 5 Mar 2010) or even medications and other therapeutic interventions. “In contrast to the genetic sequence, which is determined by inheritance and is virtually identical in all tissues, the epigenetic pattern varies from cell type to cell type and is potentially dynamic throughout life. It is postulated here that different environmental exposures. . . could impact epigenetic patterns, with important implications for mental health” (McGowan & Szyf, 2010). In contrast to genetic sequence alterations, “epigenetic alterations are potentially reversible” (McGowan & Szyf, 2010).

Depression is a likely candidate for a disease that would have epigenetic mechanisms associated with it, as there has long been a recognized connection between environmental factors and depression (Wankerl et al., 2014). If an association is found between epigenetics and depression and suicide, this could shed light on suicide prevention efforts as well as identification of biomarkers for depression that could possibly help with disease stratification.

The aim of this paper is to perform a systematic review of the literature related to the role of epigenetics and depression and suicide. We hypothesized that an association does exist.

2. METHODS

Inclusion criteria for this systematic review were studies with original data which were written in English. Exclusion criteria were review papers and papers written in languages other than English.

A search of Medline/PubMed was performed using Medical Subject Heading (MeSH) Database for articles from inception to February 23, 2015. The search terms were “Epigenomics” [Mesh] and “Depressive Disorder” [Mesh]. Of the 11 papers produced by the search, 9 were excluded because they were review papers, and one was excluded due to being a letter. The remaining study was reviewed herein.

Another search was performed using PubMed MeSH from inception to February 23, 2015. The search terms were “Suicide” [Mesh] and “Epigenomics” [Mesh]. This search yielded 9 papers, 3 of which met our inclusion criteria. The remaining 6 papers were excluded due to being review articles.

Non-MeSH searches were also performed, using the terms “epigenetics depression,” “epigenetics suicide,” “epigenetics suicidal ideation,” “epigenomics depression,” “epigenomics suicide,” and “epigenomics suicidal ideation.” When searching “epigenetics depression,” 3 additional papers were found; 2 more were found when searching “epigenetics suicide”; 1 more

was found when searching “epigenetics suicidal ideation”; and no additional papers were found when searching “epigenomics depression,” “epigenomics suicide,” or “epigenomics suicidal ideation.”

A search was conducted using the terms “(epigenetics OR epigenomics) AND (depressive disorder OR depression).” This search resulted in 164 papers. Of these, 65 were excluded due to being reviews; 1 was excluded due to being in a foreign language; 11 were excluded due to being both reviews and in a foreign language; 70 did not meet the objectives of the review, and 6 were letters. Five of the papers had already been included from other searches, and 5 more papers were found to add to the review.

A similar search of “(epigenetics OR epigenomics) AND (suicide OR suicidal ideation)” was performed, which yielded 28 papers. Of these, 9 were excluded due to being reviews; 1 was excluded due to being in a foreign language; 1 was a letter, and 83 were excluded due to not meeting the objectives of the review. Eight of the papers had already been included in the review from prior searches, and 1 new paper was added. Of note, both human and animal studies were included. See Figure 1 for more details.

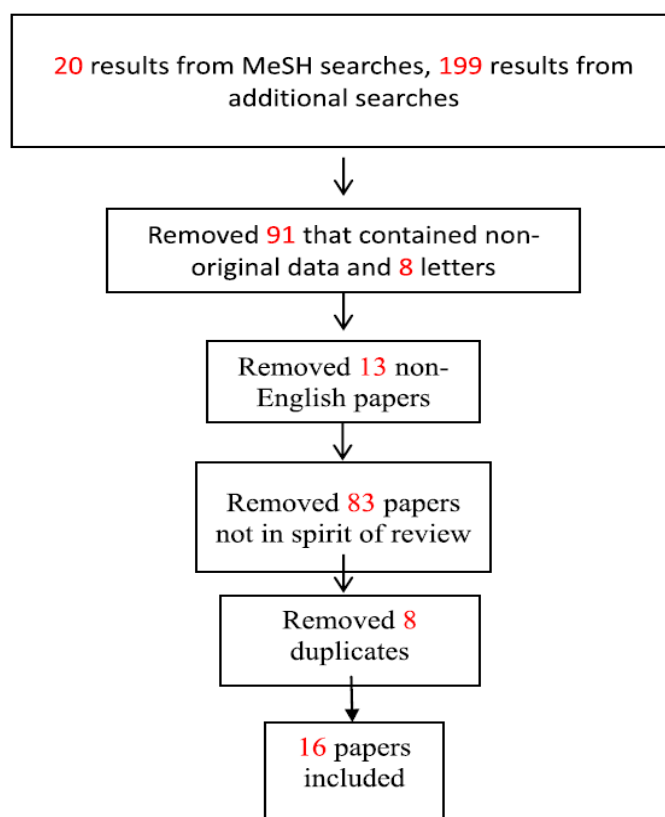


Fig. 1. Diagram of the systematic literature review of studies examining the role of epigenetics in depression and suicide.

3. RESULTS

The results of the review are summarized in Table 1 and discussed in more detail in the following paragraphs.

3.1 Epigenetics and Suicide/Suicidal Ideation

Fiori and Turecki examined postmortem brain tissue of 10 subjects who died by suicide and 10 controls (Fiori & Turecki, 2011). Cytosine-guanine dinucleotides (CpG site) methylation at Brodmann's area (BA) 8/9 showed a negative association with SAT1 expression in the suicide group. The CpG promoter as a whole, as opposed to a part, seemed to be instrumental in determining SAT1 expression. There was increased methylation at the polymorphic CpG site created by rs6526342 in the group who died by suicide, indicating that methylation at this site could mediate the association between the C allele and suicide.

Chromatin modifications were assessed to see how they affected SAT1 expression. H3K27me3 was studied as it seems to be unaffected by postmortem changes to the brain. However, there seemed to be no significant changes in H3K27me3 levels between the control group and the group who died by suicide, nor did the level of H3K27me3 seem to correlate with the expression of SAT1. A correlation was found between H3K27me3 and SAT1 exon methylation, which may be incidental, as it did not seem to make a difference in the expression of SAT1.

This study is valuable for its preliminary results of epigenetic changes in patients who died by suicide. However, there are several potential limitations to consider in regards to this study. First, the small sample size limits the power of the data analysis. Second, the study only included males, and therefore it is unclear if the results are generalizable to females. As the authors point out in their study, SAT1 is found on the X chromosome, and there is increased CpG methylation in females regardless of other factors. Finally, it is possible that other histone modifications which were not studied may be involved in important epigenetic changes.

Gross et al (2013) examined BA44 in postmortem brain tissue in 34 people who had died by suicide and 34 control subjects. The first thing that was examined was the level of gene expression of OAZ1, OAZ2, AMD1, and ARG2. All of these had increased levels of expression in the group of patients who died by suicide. Then, methylation level in the promoter regions of these genes was assessed. The mean methylation level was 3-8%. OAZ1 was the only gene to show significant methylation differences between the two groups. OAZ2 showed a trend toward significance. While there was hypermethylation of specific CpGs of promoters for both OAZ1 and OAZ2, neither of these was correlated with gene expression.

Alternatively, for AMD1 there was hypomethylation in patients who died by suicide at CpG9 and CpG 16 that showed a negative correlation with gene expression. For ARG2, hypomethylation of CpGs 5-7 in patients who died by suicide was negatively correlated with gene expression.

The researchers in this study accounted for age, post-mortem interval, and if the subject was on psychiatric medications at the time of death. As these are all possible confounding variables, it is a strength of the study that these were taken into account when processing their data. Limitations, including the small sample size, might have affected the power of the study. Also, only Caucasian males were included in the study which limits the generalizability of the findings to the wider population. Finally, it is possible that, since only a limited area (500 base

pairs) was studied, the results found were from changes further upstream than the area under investigation in this study.

Ernst et al. (2009) examined TrkB-T1 methylation in patients who died of suicide as well as other causes. All subjects were French Canadian males with postmortem interval less than 48 hours. There were 28 subjects who died by suicide and 11 subjects who died of other causes. This study used extreme value analysis to find abnormalities. The researchers initially focused on BA 8/9 and found that there was reduced TrkB-T1 expression in 10 of 28 subjects who died of suicide. Then, the researchers examined 8 other frontal cortical regions, and they found that there was similarly low TrkB-T1 expression in these subjects in all these regions. They also examined the cerebellum but did not find a decreased expression of TrkB-T1. Interestingly, the lower TrkB-T1 expression was associated with 2 particular CpG sites in the TrkB promoter.

The authors of this study (Ernst et al., 2009) investigated whether PMI, pH, or age affected TrkB-T1 expression, and found that they did not. A strength of this study is that the authors examined multiple brain regions in the same subjects and found that the effect held. The researcher also examined if a history of alcohol or cocaine abuse affected the TrkB-T1 expression, but no effect was found. A major weakness of this study is the small number of subjects. Another concern is that the statistical method utilized, the extreme value analysis method, is a new method, and it is unclear if the results are as reliable.

In a study by Keller et al. (2011), post-mortem brain tissue from 18 subjects who died by suicide and 18 control subjects were examined, with samples collected from Wernicke's area. Contrary to studies which showed a downregulation of TrkB-T1 transcript levels in other brain areas, in this study there was no significant difference in TrkB-T1 expression and TrkB promoter methylation in Wernicke's area between the control group and the group of patients who died by suicide. The authors also extrapolated previously obtained data about BDNF promoter methylation from a previous study they had performed as well as subjects in this study, and found that the BDNF promoter was hypermethylated in patients who died of suicide; these data are in accordance with that of Kang et al. (2013) and Kim et al. (2014).

An important aspect of this study (Keller et al., 2011) is that it examined epigenetic findings in suicide in an area that has not been investigated before: Wernicke's area. Although the study had a small sample size and was preliminary in nature, it provided a base for further studies in this area (Fiori & Turecki, 2011; Gross et al., 2013; Maussion et al., 2014). The results may not be generalizable given that only Caucasian individuals were studied.

Maussion et al. (2014) examined the epigenetics of suicide in BA 8/9 involving TrkB. Other than looking at the promoter region, this study investigated the methylation in other TrkB gene regions. A methylation study was done on 11 subjects who died by suicide and who had low TrkB-T1 levels plus 13 subjects who died of other causes and who were not selected on the basis of TrkB-T1 level. Next, genotyping of the rs1624327 variant was performed on the 24 subjects that were involved in the methylation study, as well as 22 additional subjects who died by suicide and who also had low TrkB-T1 levels, plus 15 additional subjects who died of other causes and were not selected on the basis of TrkB-T1 levels. All subjects were male French Canadians and were matched according to age and postmortem interval.

In the microarray analysis of the 11 subjects who died by suicide and had low TrkB-T1 levels and 13 subjects who died of other causes and were not selected on the basis of TrkB-T1 level, five consecutive probes were found to be hypermethylated in the subjects who died by suicide. The 1 kb region that encompassed these five probes was located at the TrkB 3' UTR and contained 13CpGs. In this region, there was overall significant hypermethylation in the group who died of suicide versus the group who died of other causes. This was further broken down to indicate that the hypermethylation was clustered in CpGs6-9. From the expanded sample for rs1624327, no significant differences were found between the group that died of suicide and the group that died of other causes.

This study (Maussion et al., 2014) had some strengths, including that it built on the data of a previous study to further elucidate and differentiate the data. There were also some weaknesses, including the small sample size, especially of the microarray analysis. Additionally, the data included only males of French Canadian origin, which makes it difficult to generalize the results to a more diverse population.

Kang et al. (2013) investigated BDNF promoter methylation in patients with suicidal ideation. They examined 108 patients with a diagnosis of major depressive disorder who completed 12 weeks of outpatient evaluations. The patients involved in the study received treatment "as usual" by their psychiatrists. The patients were treated with nine antidepressants including escitalopram, paroxetine, sertraline, fluoxetine, bupropion, mirtazapine, venlafaxine, amitriptyline, and imipramine. The patients were questioned about a previous suicide attempt as well as suicidal ideation at baseline and at weeks 1, 2, 4, 8, and 12 of treatment. The Beck Scale for Suicidal Ideation was administered at baseline and at weeks 1, 2, 4, 8, and 12. Several other ratings scales were also used to quantitatively assess depressive symptoms.

There was significantly higher BDNF methylation in males, as well as higher BDNF methylation associated with lower scores on the Social and Occupational Functioning Assessment Scale (SOFAS) and World Health Organization Quality of Life Abbreviated Form (WHOQOL-BREF). No association between BDNF methylation and Hamilton Depression Rating Scale (HAMD) score was found, so the authors further separated the HAMD scores into <20 and ≥20, but there still was no association between HAMD score and BDNF methylation.

Of the subjects, 19.4% had previous suicide attempts, 51.9% had suicidal ideation at baseline; 52.8% had suicidal ideation during treatment, and 25% had suicidal ideation at the last treatment session. Twenty-five percent of the patients had suicidal ideation during treatment but not at baseline, and 5.6% had self-harm during treatment. Previous suicide attempt, suicidal ideation during treatment, and suicidal ideation at the last treatment session were found to be significantly associated with a higher BDNF methylation level, even when adjusted for gender, SOFAS, and WHOQOL-BREF scores. There was no significant association between antidepressant type and BDNF methylation nor between antidepressant type and suicidal outcome. Of the patients with lower BDNF methylation, there was significant improvement in Beck Scale for Suicidal Ideation scores over the 12-week treatment period. However, in patients with higher levels of BDNF methylation, there was much less improvement in Beck Scale for Suicidal Ideation scores.

This study has several strengths. First, the investigators used several scales to objectively assess symptoms. Second, the investigators statistically controlled for antidepressant use. The major weakness of this study is the lack of a control group. Of note, in this study and in some of the others reviewed, blood samples were used. There is, however, some concern about how the results of blood samples translate with postmortem brain autopsy samples.

Kim et al. (2014) investigated the methylation of BDNF promoter in a geriatric Korean population. Community residents of two areas of Kwangju, South Korea, were asked questions about suicidal ideation at baseline and were followed up at two years. Both at baseline and at the 2-year follow-up, participants were given the Geriatric Mental State Schedule, and suicidal ideation within the past month was assessed.

Other data collected were age, sex, education level, depressive symptoms, cognitive function, and any level of disability. The Geriatric Depression Scale was used to assess level of depression, the Mini Mental State Exam to assess cognitive function, and the World Health Organization Disability Schedule II to assess level of disability.

At baseline, 732 subjects were interviewed, of which 93 endorsed suicidal ideation within the past month (Kim et al., 2014). The factors that were most commonly associated with suicidal ideation were older age, lower cognitive function, more severe depressive symptoms, and higher level of disability; hence, these factors were chosen as covariates for this study. Only subjects who did not endorse suicidal ideation at baseline were chosen to be followed (639 subjects). Of these, 57 endorsed suicidal ideation within the past month at 2-year follow-up. Of the remaining subjects, 24 were lost to follow-up, 12 had died, 10 refused to participate, 8 had changed their addresses, and 6 were too unwell to participate. There were no statistical differences between the subjects who participated in the two year follow-up and those who did not.

Suicidal ideation at baseline was significantly associated with higher methylation at BDNF promoter CpGs 5, 7, 8, and 9 (Kim et al., 2014). Suicidal ideation at 2-year follow-up was significantly associated with higher methylation at BDNF promoter CpG 9.

This study (Kim et al., 2014) has several strengths. First, it had a larger sample size than many of the other studies that have been reviewed herein, which makes the power of the data stronger. It also confirmed the findings of Kang et al. that BDNF methylation is significantly associated with suicidal ideation (Kang et al., 2013). Another strength of this study is that it had a large retention rate, and there was no statistical significance between the participants who followed up and those lost to follow-up. Additionally, there are few studies that have focused on the geriatric population in the area of epigenetics and suicidality. A weakness of the study is that it did not utilize a control group. Also, the study (Kim et al., 2014) only utilized one CpG island to assess for BDNF methylation status (similar to (Kang et al., 2013)).

Klempan et al. (2009) examined QKI gene expression and epigenetics in subjects who had died of suicide and who had major depressive disorder. The researchers assessed 16 patients who had died of suicide and 13 controls. The following brain areas were examined: BA 4, 6, 8/9, 10, 11, 20, 21, 24, 29, 38, 44, 45, 46, and 47 as well as the hippocampus, amygdala, and nucleus accumbens. The number of subjects varied by brain region, with the median for the suicide group being 10 and the median for the control group being 7. The researchers found that the QKI

expression was significantly downregulated at most areas tested (BA4, BA8/9, BA11, BA20, BA21, BA44, BA45, BA46, and BA47). Five of the subjects had a history of alcohol abuse, and this was not found to be related to the QKI expression. The researchers had hypothesized that the difference in QKI expression was due to epigenetic changes, but found little methylation at the promoter for QKI in either group.

Previous studies had shown decreased QKI expression in schizophrenia ((Aberg et al., 2006) and (Haroutunian, Katsel, Dracheva, & Davis, 2006)), and this study further extended that work in subjects with major depressive disorder. A limitation of this study is its small sample size. Further, it is difficult to tease out if the decrease in QKI expression was due to the history of major depressive disorder or suicidality.

Haghighi et al. (2014) examined DNA methylation in 25 subjects who died of suicide (and had major depressive disorder) as compared to 28 subjects who died of other causes (with no axis I DSM-IV diagnosis). All subjects had negative toxicological screening for psychotropic or illicit drug use in the last 3 months. The researchers tested BA47 for DNA methylation at 27,578 CpG sites. The data showed that all CpG sites had an increase in DNA methylation with age. However, in the group that died by suicide, the increase in methylation was 8 times greater.

This study has several strengths. First is the large amount of CpG sites tested. Second, the researchers excluded patients with psychotropic or illicit drug use three months pre-mortem, which eliminated a potential confounding factor. Weaknesses include that only one brain area was tested and the small sample size. Also, increased methylation may be hard to discern if related to depression or suicide.

Nagy et al. (2015) examined epigenetic changes in astrocyte marker expression in the context of patients who have died of suicide. The researchers examined 76 subjects who died of suicide and 45 controls. Using BA10, they identified males within the lowest quartile of expression of seven genes: GFAP, ALDH1L1, SOX9, GLUL, SLC1A3, GJA1, and GJB6 (for a total of 22 subjects). Then, the control subjects were picked in order to best match the characteristics of the suicide group. Methylation analysis was done of the genome and differentially methylated regions (DMRs) were identified. The researchers chose to focus on two DMRs, GRIK2 and BEGAIN, that are important in astrocyte function. They found that GRIK2 had an increased expression in the patients who had died of suicide. For BEGAIN, there are two transcripts, but for variant 1 (which is expressed mostly in the brain), they found a decreased expression. The researchers then separated tissue into neuronal and non-neuronal fractions and found no difference for GRIK2, but found an increase in methylation in the non-neuronal fraction for BEGAIN.

This study is one of the first to use next-generation sequencing to investigate epigenetic changes, and it is a very innovative approach to investigating astrocytic epigenetic changes. One weakness is that the authors state that their data apply to subjects with depression and suicide; however, subjects with a DSM-IV diagnosis of major depressive disorder as well as with no axis I diagnosis were included in the study group. Also, a low number of patients were studied and, since the study was restricted to males, it may not be applicable to females.

3.2 Epigenetics and Depression

A study by Dell'Osso et al. examined the difference in BDNF promoter methylation in subjects with major depressive disorder (MDD), bipolar I disorder, bipolar II disorder, and healthy control subjects (Dell'Osso et al., 2014). The study examined 43 subjects with major depressive disorder, 61 subjects with bipolar I disorder, 50 subjects with bipolar II disorder, and 44 age-matched healthy controls. The patients with psychiatric diagnoses were on stable pharmacologic treatment.

There was a similar level of BDNF DNA methylation in the MDD patients as in the bipolar II patients, both significantly more than the bipolar I patients. The authors also stratified data according to the mood state of the patients and found that BDNF DNA methylation of manic/mixed patients was comparable to healthy controls, whereas BDNF DNA methylation of depressed patients was comparable to euthymic patients. Methylation was significantly higher in depressed patients compared to manic/mixed patients. BDNF DNA methylation was found to be lowest in bipolar I disorder patients regardless of mood state as compared to patients with major depressive disorder and bipolar II disorder. Patients with bipolar II disorder in a euthymic and depressed state were found to have a higher level of BDNF DNA methylation compared to bipolar II patients in a hypomanic state.

BDNF DNA methylation was analyzed on the basis of the type of pharmacologic treatment that the patients were receiving. It was shown that, overall, lithium and valproate tended to decrease the level of methylation at the BDNF gene promoter as compared to other classes of medications (antidepressants, antipsychotics) but that this decrease was not of a statistically significant level.

There are several strengths of this study. The study identifies an epigenetic marker for depression, and further stratifies it according to manic versus depressive phases of bipolar disorder. The authors of the study also included potential confounding variables (pharmacological treatment, age, and sex) in the statistical model.

Melas et al. (2012) examined epigenetic effects associated with depression in rat models. The study examined prefrontal cortices of Flinders Sensitive Line (FSL) and Flinders Resistant Line (FRL) rats. Control rats were used as well as rats that had been treated with escitalopram from both lines. The escitalopram was administered for 3 weeks prior to the euthanasia of the rats. There were 12 FRL rats, 14 FSL, 6 FRL treated with escitalopram, and 9 FSL treated with escitalopram.

P11 mRNA has previously been shown to specifically interact with serotonin levels in the prefrontal cortex. The investigators assessed if there was a difference in P11 mRNA expression between the control FSL and FRL rats. There was significantly reduced P11 mRNA expression in the pre-frontal cortices of the FSL rats (which served as the model for depression). This was confirmed by Western blotting which showed decreased protein expression. The FSL rats showed hypermethylation in the P11 promoter compared to the controls. *In silico* analysis revealed that two CpG sites in the P11 promoter region were located within a putative binding site of the androgen receptor (AR), a DNA-binding transcription factor (TF) that inhibits corticotropin-releasing factor (CRF). Reduced AR mRNA levels have previously been reported in post-mortem brains of depressed individuals (Wang et al., 2008). However, Melas et al. did not find significant

difference of AR mRNA levels between FSL and FRL rats, suggesting that aberrant AR mRNA levels may not underlie the depressed phenotype in this rat model.

In the escitalopram-treated rats, P11 mRNA levels were significantly higher when compared to the rats left untreated. In fact, the P11 of FSL rats treated with Escitalopram was found to be at a level that was not statistically different from the P11 level of the FRL rats.

Similarly, in the FSL rats treated with escitalopram, the level of methylation of the P11 promoter was statistically undifferentiated from the FRL group.

The mRNA levels of three DNA methyltransferases as well as four genes encoding proteins were examined to see if they are involved in the methylation changes observed. Of the methyltransferases examined (Dnmt1, Dnmt3a, Dnmt3b), Dnmt1 and Dnmt3a were significantly decreased after escitalopram treatment in the FSL group. Of the genes examined, there was no significant difference for Mbd2, Mbd4, or Gadd45b, though Gadd45b did show a trend toward significance.

As a rat study, this study occurred in a much more controlled environment than the other studies reviewed herein. On the other hand, weaknesses of the Melas et al. study may include the lack of applicability of this rat study to humans.

Davies et al. (2014) investigated human discordant twin pairs for major depressive disorder to identify epigenetic changes associated with the disorder. The study included two samples, 27 monozygotic twin (MZT) pairs from the UK and 23 MZT pairs from Australia.

The two data sets from the twin pairs were integrated through a meta-analysis (Davies et al., 2014). Genes identified in the meta-analysis as hypermethylated were evaluated in conjunction with existing literature to select for likely hypermethylated regions associated with major depressive disorder. In order to replicate these findings, blood MeDIP-seq data from 354 unrelated, age-matched females from the EpiTwin project were included, 118 suffering from major depressive disorder and 236 controls. Four DMRs (differentially methylated regions) were selected for replication. They were also compared with an independent dataset of 932 postmortem brain samples from the Edinburgh Brain Bank, which included 932 brain samples of ten different brain regions obtained from 101 unrelated individuals (24 male and 77 female) ages 16 to 83. The brain regions were cerebellum, frontal cortex, hippocampus, medulla, occipital cortex, putamen, substantia nigra, temporal cortex, thalamus, and intralobular white matter.

The meta-analysis of the UK and Australian data revealed 17 DMRs of genome-wide significance. Four of these were in genes related to the pathology of major depressive disorder: ZBTB20, AGTPBP1, TBC1D8, and CLSTN1. Each of these was replicated in an independent replication cohort of 354 unrelated age-matched females, and there was 28.2% increased methylation in the depressed cohort compared to the control group. In the meta-analysis, ZBTB20 was the second most differentially methylated region (of all seventeen DMRs identified).

The investigators made two additional linear mixed models with the UK data subset, as described above, in order to rule out antidepressant medication as a confounding factor for the results found, and it was found that ZBTB20 is highly methylated in subjects with major depressive disorder regardless of whether they are taking antidepressant medications.

Additionally, examination of 932 postmortem brain samples from the Edinburgh Brain Bank from ten different brain regions found that ZBTB20 is highly expressed in the hippocampus, cerebellum, and white matter regions, and lowly expressed in the frontal, occipital, and temporal cortices.

A strength of this study (Davies et al., 2014) is that the researchers accounted for several confounding variables, including age, BMI, smoking status, and antidepressant medication. A weakness is that subjects from the two twin sets were diagnosed using different questionnaires, which could be a confounding factor. Additionally, the majority of the twin pairs studied were female, which limits the generalizability of the results for males.

Weder et al. (2014) investigated the effects of child abuse on epigenetics and depression. The study included 94 maltreated children and 96 healthy control children with no history of maltreatment or exposure to intrafamilial violence and no past psychiatric illness. The maltreated children had been removed from their parents' care. Many objective measures were used to assess diagnoses for the subjects. Of the maltreated children, 50% met criteria for PTSD, 12% for major depressive disorder, 17% for dissociative disorder and 17% for dissociative disorder not otherwise specified (Weder et al., 2014). Of the children with depressive disorder, 88% were comorbid for PTSD.

The maltreated children in the study had a range of 1-7 instances of substantiated abuse or neglect, with an average of 3.92% of the children experienced more than one type of abuse or neglect: 65% of the children had experienced physical abuse, 24% sexual abuse, 83% neglect, 65% emotional abuse and 70% had witnessed domestic violence. The Mood and Feelings Questionnaire (MFQ) was used to assess the children's depression symptomatology. Twenty-six percent of maltreated children and 4% of controls met the threshold for depression (score of 27).

Three genes (ID3, TPPP, and GRIN1) had methylation values at CpG sites that were significantly related to depression scores. Greater methylation at the CpG sites (related to ID3, TPPP, and GRIN1) were associated with lower depression scores. Methylation changes at these genes appeared to be independent of maltreatment history, age, race, and gender. Additionally, methylation changes appeared to be independent of whether the children met criteria for a PTSD diagnosis.

One strength of this study is that it had two quality control measures to ensure that the data were of high standards. Additionally, the researchers employed several objective measures to diagnose their subjects. Weaknesses include that the study included only children, thus limits generalizability to adults, and the inclusion of siblings may have led to unintentional bias.

Melas et al. (2013) examined genetic and epigenetic associations of monoamine oxidase A (MAOA) gene and glucocorticoid receptor (NR3C1) gene with childhood adversities and the later development of depression. The researchers used data from the PART study, which is a longitudinal population-based mental health study of Swedish citizens. They investigated the relationship between childhood adversities, depression, and MAOA gene alleles. Childhood adversities were associated with depression in both males and females. However, females who experienced childhood adversity and had the MAOA-L allele were at higher risk than those with MAOA-H allele. There was no difference between these subtypes in men. Also, in MAOA-L

females, exposure to one childhood adversity was associated with higher risk for depression. This association was less pronounced in males.

The researchers (Melas et al. 2013) then assessed the following childhood adversities to see if they altered methylation levels of MAOA and NR3C1 in females with depression and controls: parental death, parental divorce, financial problems, and familial constraints. They found a hypomethylation pattern of the MAOA in depressed females compared to controls and that this association was independent of the MAOA genetic variations. In addition, an association of early parental death with hypermethylation of the NR3C1 was found in females. However, this association was found only among MAOA-L but not MAOA-H, indicating that the MAOA-genotypic variations may affect the extent of NR3C1's epigenetic programming as a result of this traumatic experience.

This study (Melas et al., 2013) analyzed data from the PART study, which included 8613 randomly selected individuals. However, a subset analysis of NR3C1 included only 10 females with depression and early parental death and only 2 controls, thus making the data difficult to interpret due to its lack of power. The PART study also used saliva to collect DNA, which is unique among the studies reviewed herein, and though the researchers assert that saliva shows directly what is in blood leukocytes, this is not yet a commonly used method. The DNA methylation portion of the study included females, thus it is not generalizable to males. Another limitation in the generalization of the PART study is that it only examined the Swedish population, and the study has not been replicated in other samples.

Numata et al. (2015) examined medication-free patients with major depressive disorder in an effort to isolate biomarkers. To this end, the researchers had two parts to their study: discovery and replication. For their discovery sample, they had 20 medication-free patients with major depressive disorder (2 males and 18 females, only one patient who was on zolpidem) and 19 medication-free control subjects with no mental illness. For the replication study, they had 12 medication-free patients with major depressive disorder (3 males and 9 females, one patient was taking tandospirone) and 12 medication-free controls with no mental illness. The researchers looked for differences in DNA methylation between the two groups at 431,389 CpG sites, and differences were found at 363 sites. Of the 363 sites, all had lower methylation in the patients with major depressive disorder as compared to the controls, and notably this included some CpG sites which had previously been hypothesized to be associated with major depressive disorder, including CITED2, DGKH, GSK3B, and SGK1. The researchers then did a replication study. In this study, of the top 100 methylated CpG sites in the discovery arm, 84 were significant.

This study took a large leap forward in trying to identify epigenetic biomarkers. There were many CpG sites investigated. The authors also did both a discovery and replication step, which further validates the data. Limitations included the small number of subjects, and the much higher percentage of females.

Table 1
Studies examining the role of epigenetics in depression and suicide.

Studies	Subjects	N	Brain area	Gene(s) targeted	Epigenetic difference found?	P
Fori and Tur-echi (2011)	Human	10	BA 8/9	SAT1	Yes	One-tailed $p = 0.07$
Gross et al. (2013)	Human	34	BA 44	OMZ1, OMZ2, AMD1, ARG2	Yes	OMZ1 $p = 0.035$, OMZ2 $p = 0.041$, AMD1 $p = 0.025$, ARG2 $p = 0.027$
Ernst et al. (2009)	Human	28 Patients who died by suicide, 11 controls	BA 8/9, 4, 6, 10, 11, 44, 45, 46, 47, cerebellum	TRBT1	Yes	BA 8/9 $p = 0.003$; p for BA 11, 44, and 45 < 0.01
Keller et al. (2011)	Human	18	Wernicke's area	TRKB region	No	No significant findings found
Mausson et al. (2014)	Human	11 Subjects who died by suicide and 13 controls for methylation analysis; 33 subjects who died by suicide and 26 control subjects for genotyping	BA 8/9	TRKB-T1	Yes	$p = 5.7 \times 10^{-4}$
Kang et al. (2013)	Human	108	Peripheral blood	BDNF	Yes	BDNF methylation with previous suicide attempt $p = 0.035$, BDNF methylation with suicidal ideation during treatment $p = 0.013$, BDNF methylation with suicidal ideation at last treatment period $p = 0.039$
Kim et al. (2014)	Human	732	Peripheral blood	BDNF	Yes	Suicidal ideation prevalence: CpG 5 $p = 0.002$, CpG 7 $p = 0.003$, CpG 8 $p \leq 0.001$, CpG 9 $p \leq 0.001$; suicidal ideation incidence CpG 9 $p = 0.002$
Klumpen et al. (2009)	Human	16 Subjects who died by suicide with major depressive disorder, 13 control subjects. Number of subjects for each area varied due to quality control measures	BA 4, 6, 8/9, 10, 11, 20, 21, 24, 29, 38, 44, 45, 46, 47, hippocampus, amygdala, and nucleus accumbens	QKI	No	BA 4 $p = 0.013$; BA6 $p = 0.506$; BA 8/9 $p = 0.38$; BA 10 $p = 0.463$; BA 11 $p = 0.023$; BA 20 $p = 0.032$; BA 21 $p = 0.068$; BA 24 $p = 0.436$; BA 29 $p = 0.657$; BA 38 $p = 0.294$; BA44 $p = 0.011$; BA 45 $p = 0.020$; BA 46 $p = 0.028$; BA 47 $p = 0.005$; amygdala $p = 0.234$; hippocampus $p = 0.155$; nucleus accumbens $p = 0.238$ $p < 2.2 \times 10^{-6}$
Haghighi et al. (2014)	Human	25 Subjects who died by suicide with major depressive disorder, 28 controls	BA47	Methylation at 15,249 CpG sites	Yes	CR1K2 $p < 0.001$; BECAIN $p < 0.001$
Nagy et al. (2014)	Human	22 People who died by suicide, 22 controls	BA10, replication with BA 8/9	GRK2 and BECAIN	Yes	BDNF methylation significantly higher in MDD and bipolar II compared to bipolar I $p < 0.01$; BDNF methylation significantly higher in depressed state compared to manic/mixed $p < 0.01$
DeLillo et al. (2014)	Human	44 Subjects with major depressive disorder, 61 with bipolar I disorder, 50 with bipolar II disorder, and 44 controls	Peripheral blood	BDNF	Yes	FSL vs. FSL-Esc $p = 0.002$
Melas et al. (2012)	Rat	Variable—12 FRL, 12 FSL, 6 FRL treated, 9 FSL treated	Prefrontal cortex	P11	Yes	$p = 0.0487$
Davies et al. (2014)	Human	50 Twin pairs	Peripheral blood	ZBTB20	Yes	ID3 $p = 0.005$; TRPP $p = 0.02$; GRIN1 $p = 0.03$
Weder et al. (2014)	Human	94 Maltreated children, 96 healthy controls	Saliva	ID3, TRPP, GRIN1	Yes	Female gender and childhood adversity association with MAOAO $p = 0.003$; hypermethylation of CpG 35 with early parental death in subjects with depression $p = 0.005$
Melas et al. (2013)	Human	82 Subjects with depression, 92 controls	Saliva	MAOA and NR3C1	Yes	For CSK38 $p = 9.7 \times 10^{-6}$ for discovery set and 1.4×10^{-4} for replication set
Numata et al. (2015)	Human	Discovery phase 20 subjects with major depressive disorder and 19 controls; replication phase 12 subjects with major depressive disorder and 12 controls	Peripheral blood	Methylation of 431,489 CpG sites, including CSK38	Yes	

4. DISCUSSION

This paper reviewed the role of epigenetics on depression and suicide. Our hypothesis was that depression and suicide are associated with epigenetics alterations, which was supported by the majority of papers reviewed.

Maussion et al. (2014) examined methylation at the TrkB-T1 region. They found significant hypermethylation at this area in subjects who died of suicide in comparison to subjects who died of other causes. The TrkB region was also examined in the Keller study (Keller et al., 2011), which did not find any difference in methylation between patients who died of suicide and patients who died of alternative means.

Ernst et al. (2009) also examined the TrkB region in postmortem brain tissue of control subjects and subjects who died of suicide, both in BA8/9 as well as eight other cortical regions and the cerebellum. In the Ernst paper, a difference in TrkB methylation specific to the TrkBT1 site was found in the group of people who died by suicide. The researchers in the Ernst study did not look at Wernicke's area, which is BA22.

There are several possible reasons for the differences in these findings. First, both Ernst and Maussion examined the BA8/9 region, while Keller examined Wernicke's area. It is possible that the methylation difference found in people who died of suicide is not present in all regions of the brain. Second, all three studies had small sample sizes (Table 1). It is possible that none of these studies were powered adequately to give definitive findings. Additionally, the Ernst and Maussion studies were both performed by the same laboratory, and it is possible that some of the subjects could have been overlapping (other papers in this review performed by that laboratory include Fiori and Turecki (2011), Gross et al. (2013), Klempan et al. (2009), and Nagy et al. (2015). It is conceivable that some of the studies reviewed herein from this laboratory had overlapping samples, but it is impossible to tell this with certainty (personal correspondence with Dr. Turecki).

BDNF methylation was found to be significantly associated with suicidal ideation by Keller et al. (2011), Kang et al. (2013) and Kim et al. (2014). The Dell'Oso study (Dell'Oso et al., 2014) further examines when BDNF is hypermethylated in psychiatric illness as well as current treatments that help to decrease this methylation. Dell'Oso et al. (2014) found that BDNF methylation in patients with major depressive disorder was higher than in patients in a manic or mixed state of bipolar disorder. Further, they found that while lithium and valproate decreased the level of BDNF methylation, the decrease was not statistically significant.

This review paper has some weaknesses, in that the studies reviewed were all preliminary in nature and had small sample sizes. Nonetheless, most studies were in agreement concerning the association between depression and suicide and epigenetic alterations. Another possible limitation of our study is publication bias. It is possible that researchers who did not find a role for epigenetics in depression and suicide may not have published their studies. Another limitation is the possible overlap of samples in six of the papers reviewed, which could account for some of the results seen.

However, the agreement among most of the reviewed studies is highly suggestive that epigenetics may play an important role in depression and suicide. This can provide an important

biomarker in predicting disease state and disease stratification that might help move the field forward towards precision medicine. Moreover, as epigenetic modifications are functional modifications, rather than structure changes in the genome, epigenetic modifications may offer an easier and more effective mode for future therapeutic methods. Thus, further studies to better characterize epigenetic modifications are promising. Epigenetics has a promising role in the future for both new therapeutic avenues and as a possible psychiatric biomarker, but more research in this field is needed.

Contributions (added for the purpose of this thesis): Nagy Youssef conceptualized the idea of the manuscript, developed the inclusion criteria and search strategy, and mentored Laura Lockwood, his resident physician at the time of this systematic review. They searched the literature, and both contributed equally to the text and edited the paper of the initial draft. Shaoyong Su provided input on the later draft in conceptualization and editing, and all coauthors approved the final version of the manuscript. Also, of note, none of the chapters used in this thesis, to my knowledge, have been used for any other PhD thesis.

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Chapter 3 The Effects of Trauma, With or Without PTSD, on the Transgenerational DNA Methylation Alterations in Human Offspring

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Abstract

Exposure to psychological trauma is a strong risk factor for several debilitating disorders including post-traumatic stress disorder (PTSD) and depression. Besides the impact on mental well-being and behavior in the exposed individuals, it has been suggested that psychological trauma can affect the biology of the individuals, and even have biological and behavioral consequences on the offspring of exposed individuals. While knowledge of possible epigenetic underpinnings of the association between exposure to trauma and risk of PTSD have been discussed in several reviews, it remains to be established whether or not trauma-induced epigenetic modifications can be passed from traumatized individuals to subsequent generations of offspring. The aim of this paper is to review the emerging literature on the evidence of transgenerational inheritance due to trauma exposure on the epigenetic mechanism of DNA methylation in humans. Our review found an accumulating amount of evidence that the enduring effect of trauma exposure can be passed down to offspring transgenerationally (via the epigenetic inheritance mechanism of DNA methylation alterations), which has the capacity to change the expression of genes and the metabolome. This manuscript summarizes and critically reviews the relevant original human studies in this area. Thus, it provides an overview of where we stand, and a clearer vision of where we should go in terms of future research directions.

Keywords

psychological trauma; post-traumatic stress disorder; epigenomics; DNA methylation; prevention; treatment; transgenerational effect; transgenerational inheritance

1. INTRODUCTION

Post-traumatic stress disorder (PTSD) is characterized by four symptom clusters as defined by the Diagnostic and Statistical Manual, Fifth Edition (DSM-5) (American Psychiatric Association, 2013): re-experiencing, avoidance of stimuli associated with the trauma, negative cognitions and affect associated with the trauma, and hyperarousal symptoms and signs. Fifty to 85% of Americans experience at least one traumatic event during their lifetimes, but only 7.8% go on to develop PTSD (Kessler & Wang, 2008). Thus, the question that frequently arises is: why do some people develop PTSD after experiencing trauma, while others do not? Possible explanations could be: an underlying genetic or epigenetic risk in those who are more prone to develop PTSD and/or a protective (epi)genetic makeup, or some form of high psychological resiliency in those who do not develop PTSD or who quickly recover from PTSD, i.e., resilient individuals.

PTSD has long been established to be due to exposure to trauma, and it has been assumed that only environmental factors could contribute to the development of PTSD. On the other hand, heritability of PTSD has been estimated to be between 30% and 70% in twin studies (Sartor et al., 2012; Sartor et al., 2011; Stein, Jang, Taylor, Vernon, & Livesley, 2002; True et al., 1993; Xian et al., 2000). Genetic studies of both candidate genes and genome-wide association studies (GWAS) have provided many interesting and promising findings, yet (so far) no robust genetic variants for PTSD have been identified (Logue et al., 2015). This risk is unlikely to be fully explained by only structural genetics (Ratanatharathorn et al., 2017).

Thus, the field of epigenetics could offer insights into differential susceptibility of risk to develop psychopathology. According to Goldberg et al. (Goldberg, Allis, & Bernstein, 2007), epigenetics is the study of functionally stable and ideally heritable changes in gene expression or cellular phenotype that occurs without changes in base pairing. In other words, epigenetics involves functional changes to the gene without sequence changes. The best-studied epigenetic mechanism so far is via DNA methylation. We define DNA methylation as the attachment of methyl groups to the DNA molecule. When methyl groups are attached to the promoter, they typically act to repress gene transcription.

DNA methylation changes in genes have been associated with several psychiatric dysfunctions. For instance, we previously found an association between DNA methylation and both depression and suicide (Lockwood, Su, & Youssef, 2015). Hypermethylation of BDNF promoter or TrkB were especially involved in suicide (Lockwood et al., 2015). Another equally interesting area of research is the epigenetic changes associated with PTSD.

Only a few reviews have thoroughly examined this area of the literature. For instance, a review by Ramo-Fernandez et al. (Ramo-Fernandez, Schneider, Wilker, & Kolassa, 2015) focused primarily on the epigenetic changes associated with war trauma and childhood maltreatment. Also, a helpful review by Vinkers et al. discussed the effect of trauma on DNA methylation changes in humans and categorized the studies reviewed according to the timing of trauma exposure throughout the life span (Vinkers et al., 2015). The authors pointed out “there are significant drawbacks in the existing human literature” including “lack of longitudinal studies, methodological

heterogeneity, selection of tissue type, and the influence of developmental stage and trauma type on methylation outcomes” (Vinkers et al., 2015). A recent longitudinal study by Rutten et al. examined the genome-wide blood DNA methylation profile changes as associated with the development of PTSD symptoms over time in two military cohorts (discovery, $N=93$, and replication data sets, $N=98$) (Rutten et al., 2018). The researchers found that development of PTSD symptoms over time in combat soldiers was significantly associated with DNA methylation changes (Rutten et al., 2018). The researchers suggested that the DNA methylation mediated the relation between combat trauma and PTSD symptoms longitudinally (Rutten et al., 2018).

Another equally important idea that has yet to be explored is that epigenetic modifications may mediate the impact of traumatization of parents to be passed to their offspring. Evidence from epigenetic cell and animal studies has spurred this understanding. In addition, initial recent studies in humans support these notions. The best studied of all the epigenetic mechanisms is the DNA methylation mechanism.

Since trauma and PTSD models in animals may not be well characterized (and it is not very clear how much of the animal knowledge could translate to humans), we find that in this case human studies could provide valuable insight in this area. Despite the limited literature to date in humans, a review of the transgenerational heritability as it pertains to trauma will provide both clinicians and researchers with an overview on where we stand and a clearer vision of where we should go in terms of future research directions. Transgenerational epigenetic transmission is defined as the transmission of genomic information (in this paper, DNA methylation) from one generation to the next without changing the main structure of DNA (i.e., nucleotide sequence).

Also, it should be noted that there are 2 subtypes of studies reviewed below, which involve transgenerational effects of DNA methylation changes. In the first subtype, the samples of mothers were pregnant during the time of trauma. In the second subtype, the mothers were not pregnant, and the trauma occurred before pregnancy. It seems for the latter, methylation changes to the mothers’ DNA is a necessity for the transmission to occur transgenerationally. On the other hand, when trauma occurs during pregnancy, through methylation changes, the changes in the mothers are (in theory) not a necessity for the changes in the offspring to occur. While the mechanism and intensity of transgenerational genomic transmission (among other factors) may differ between these types of studies, the field has yet to clarify these differences in future research.

It is still unclear whether trauma-induced epigenetic modifications can be passed from traumatized individuals to subsequent generations of offspring. Thus, the key question of this paper is whether trauma-induced DNA methylation modifications can be passed from traumatized individuals to subsequent generations of offspring. The aim of this paper is thus to review the emerging literature on the transgenerational effects of trauma on DNA methylation in humans and to provide insights from the current literature (PubMed was reviewed from inception until March 2018), then end with recommendations for future research directions.

2. STUDIES ON TRANSGENERATIONAL EFFECT OF TRAUMA AND PTSD

Perroud et al. (Perroud et al., 2014) examined the impact of the Tutsi genocide on the children of the women who were pregnant while genocide was ongoing in Rwanda (which took place in 1995). In 2011, more than 20% of the Rwandan population met criteria for PTSD (Munyandamutsa, Mahoro Nkubamugisha, Gex-Fabry, & Eytan, 2012). The authors investigated if the risk for PTSD had been associated with epigenetic modifications in the children of women who were pregnant at the time of the genocide (Perroud et al., 2014). Twenty-five widows and their children were included in the study, as well as 25 Rwandan control women who were pregnant at the time, but who were living abroad. Peripheral blood leukocytes were obtained and methylation levels of the promoter regions of the glucocorticoid receptor *NR3C1* and the mineralocorticoid receptor *NR3C2* were examined. Cortisol levels, mineralocorticoid, and other glucocorticoid levels were also measured. As expected, both the mothers exposed to genocide and their children had significantly higher levels of PTSD and depression than the control group. They also showed higher methylation levels at exon 1F promoter of *NR3C1*, at CpG3-CpG9. Methylation at *NR3C2* was not statistically significantly different between the two groups. Mothers and children exposed to trauma had a lower cortisol level than non-exposed mothers and their children (lower cortisol levels were found to be related to PTSD (Yehuda & Bierer, 2008)). There was a negative correlation between *NR3C1* methylation and glucocorticoid levels in plasma, but no correlation was found between mineralocorticoid level and *NR3C2* methylation level (Perroud et al., 2014). This interesting study by Perroud et al. highlighted the methylation changes in exposed individuals as well as in their children (Perroud et al., 2014) and suggested that trauma-induced methylation changes in humans can be transmitted from parents to children. Limitations of the study include small sample size and possible confounding of the results. Confounding might occur due to the following reasons in this study: (1) since the 2 groups lived in different countries, unknown confounding variables that might have influenced the comparison with the exposed group might have affected the results; (2) the authors did not mention matching the exposed group versus the control group for possible confounders; (3) other confounding factors, such as health of participants at the time of their pregnancy, parenting style, alcohol consumption, tobacco use, and dietary factors could have played a role.

Yehuda and Daskalakis et al. (Yehuda et al., 2016) examined transgenerational methylation changes of Holocaust survivors on FKBP5, a moderator of glucocorticoid activity. The researchers examined 32 Holocaust survivors and their 22 offspring as well as 8 control subjects and their 9 offspring. Blood samples were obtained for quantification of FKBP5 methylation and cortisol levels. There were significantly higher FKBP5 intron 7 methylation levels in Holocaust survivors, but significantly lower FKBP5 intron 7 methylation levels in their offspring. The authors suggested that this opposite effect seen on FKBP5 intron 7 methylation levels might be attributable to biological accommodation in the offspring. More research is needed to replicate these findings in a larger cohort. Limitations of the study include its limited sample size as well as the presence of other factors which are impossible to control for in the population, such as the extreme starvation conditions of the Holocaust survivors, which could have also contributed to the effect seen. Despite the limitations of this study, it is quite a unique and informative study. If further replicated in a larger study, these findings could change the way our field conceptualizes trauma and PTSD.

3. TRANSGENERATIONAL EFFECTS OF TRAUMA AND STRESS AND PHYSICAL HEALTH

Although the main focus of the review is on the psychological and psychiatric effect of trauma transgenerationally via methylation changes, another area that could be impacted by trauma and stress (and supported by emerging and preliminary evidence) is that the transgenerational effects may not only affect psychological health but also physical health (Hao, Youssef, Davis, & Su, 2018). These physical effects are briefly demonstrated in the following studies.

A study by Mulligan et al. (2012) tested if prenatal maternal stress in the Congo population resulted in glucocorticoid receptor *NR3C1* methylation changes in the offspring (which may lead to increasing risk of adult-onset disease) (Mulligan, D'Errico, Stees, & Hughes, 2012). The researchers found that, in 25 mother-newborn dyads, there was a significant correlation between prenatal maternal stress and increased methylation levels in the promoter of the glucocorticoid receptor gene (*NR3C1*) found in the newborns. Maternal stress was also correlated with low birth weight. They suggested that increased methylation may “constrain plasticity in subsequent gene expression and restrict the range of stress adaptation responses possible in affected individuals,” thus increasing the risk for chronic diseases later in life.

For birth weight, they found that among the stressors examined, war stress has the strongest correlation, as well as the largest effect size, accounting for 35% of the variance in birth weight (Pearson’s correlation = -0.62 , $p = 0.0009$). Within all war stress variables, rape accounted for 31% of birth weight variance “and eclipses the effect of other war stressors”.

This increased methylation of the *NR3C1* gene could increase stress reactivity and could have a long-lasting effect on vulnerability to stress, trauma, and chronic disease development.

A study by Radtke et al. (2011) examined the methylation of 10 CpG sites in the promoter region of the *NR3C1* gene in mothers who suffered pre-pregnancy or during pregnancy exposure to intimate partner violence (IPV) and their children at the age of 10–19 (Radtke et al., 2011). According to the researchers, this was the first study to examine the effect of the trauma (IPV) over the long term (*i.e.*, at offspring age of 10–19 years). The IPV was assessed retrospectively using the composite abuse scale (CAS) (Hegarty, Sheehan, & Schonfeld, 1999). The study showed methylation of the *NR3C1* promoter gene of the children is influenced by their mother’s experience of IPV during pregnancy. There was no association between the mother’s *NR3C1* methylation and IPV. The authors concluded that as “these sustained epigenetic modifications are established *in utero*, we consider this to be a plausible mechanism by which prenatal stress may program adult psychosocial function”.

However, we would like to note that the relationship may or may not be causative, as this study only showed an association, and thus causation cannot be inferred based only on this study. Other limitations of this study include: the limited sample size of 25 and the retrospective recall of IPV with the possibility (though unlikely) of inaccurate recall. However, if replicated, this finding would be important, as this is the first study to show the long-term persistence (over many years) of trauma-associated methylation of the regulator gene of the HPA-axis in the offspring. Moreover, a general caution is that, although the reviewed studies in this section did not actually address the long-term physical effects directly, they are assumed based on the

results. For instance, the Mulligan et al. (2012) study only examined birth weight, which is not a long-term effect (Mulligan et al., 2012). However, low birth weight has certainly been linked to chronic health effects later in life (as has been shown in several other studies) (Hack, Klein, & Taylor, 1995; Reyes & Manalich, 2005).

4. DISCUSSION

The limited available literature in humans suggests that children of parents who have suffered from extreme trauma have methylation modifications associated with trauma and PTSD. This was also observed in studies done in different populations, as in the case of Yehuda and Daskalakis et al. (Yehuda et al., 2016) and Perroud et al. (Perroud et al., 2014). This may support the combined influence, not only of environmental trauma, but also of the biological component of PTSD risk. Moreover, this PTSD risk can be passed from generation to generation. Of interest, the environmental transgenerational effects that lead to change in DNA methylation in offspring has also been demonstrated in several animal models. For instance, dietary supplements during pregnancy was associated with increased methylation (of the Agouti coat color gene) and causes permanent change in coat color (Dolinoy, Weidman, Waterland, & Jirtle, 2006; Waterland & Jirtle, 2003), suggesting that prenatal and natal environmental interventions could induce epigenetic alterations with robust impact on stress-related disorders. These environmental interventions could range from trauma and stress to dietary and pharmacological interventions (Lockwood & Youssef, 2017).

We want to point out, however, that in the Yehuda and Daskalakis et al. (Yehuda et al., 2016) study, the DNA methylation seen in the offspring was noted to be opposite from that seen in their parents. Although this is a perplexing finding, one possibility is that it may be a compensatory mechanism, as has been seen in Holocaust survivors who suffered extreme starvation (which is another environmental factor that can affect the epigenome) and whose offspring were more prone to metabolic syndrome (Flory, Bierer, & Yehuda, 2011).

However, in line with the well-established notion that glucocorticoids are stress hormones, many of the studies reviewed found that the glucocorticoid receptor (*NR3C1*) gene is associated with methylation changes. For instance, maternal exposure to intimate partner violence during pregnancy was associated with increased *NR3C1* DNA methylation in teenage children (Radtke et al., 2011). Maternal exposure to war violence or rape during pregnancy was associated with increased methylation in the *NR3C1* promoter region in newborns (Mulligan et al., 2012; Rodney & Mulligan, 2014).

Some weaknesses of this review and the current literature include the limited number of studies, most of which had small sample size. It is also unknown if there was publication bias related to studies on this topic, where studies with negative results were not published. Also, there may have been other confounding factors which could have led to epigenetic changes that were not accounted for by the studies. Moreover, several of the studies represent cross-sectional studies, where causation cannot be inferred directly from these studies.

It should be noted that these findings are still preliminary and should be taken with caution. Thus, the findings need to be replicated in larger studies with control groups, which will

also increase our understanding, further specify, and solidify these findings. Future studies should also (as some of the studies reviewed did) clarify the timing of the trauma: whether it occurred prior to pregnancy or during pregnancy. In addition, further differences based on the trimester of pregnancy may be important to know. Also, the chronicity versus acuity of the transgenerational trauma needs to be considered and studied. Future research on this topic may lead to identification of biomarkers of trauma and PTSD risk and to greater advances into the prevention and treatment of PTSD. In addition, future studies should also follow the research recommendations of the National Advisory Mental Health Council Workgroup on Genomics (NAMHC), including using strict standards of statistical rigor for disease association, moving away from the candidate gene approaches, which have not been very fruitful, and moving towards well-powered genetic and epigenetic association studies (Gordon, 29 Mar 2018; National Advisory Mental Health Council Workgroup on Genomics, 2018). Expanding the genetic association studies beyond the DSM nosology, for instance, studying the effect of trauma *per se* on and the methylation changes that associate with the development of psychopathology in general (and not necessarily PTSD alone), would be helpful (since heritability is shared across psychiatric disorders). Replicating the finding in diverse populations and identifying the differences that may be unique to a particular population would be helpful. This will require data sharing between many centers to generate an adequate sample size as well as strong, meaningful significances in genome-wide studies as indicated also by the NAMHC (National Advisory Mental Health Council Workgroup on Genomics, 2018).

If the findings in the reviewed studies are confirmed definitively and further specified, it can both inform clinical research, as well as prove clinically beneficial in predicting risk to PTSD development as well as treatment planning.

5. CONCLUSIONS

Despite the limitations of the current literature, there seems to be accumulating evidence to suggest the transgenerational transmission of DNA methylation changes from parents to children. This area merits further replication of the presented findings. In addition, as some of the studies reviewed did, future studies should also clarify the timing of the trauma (of whether it occurred prior to pregnancy or during pregnancy), and if there are further differences based on the trimester of pregnancy. Also, the chronicity versus acuity of the transgenerational trauma needs to be considered and studied. Future research of this topic may lead to identification of biomarkers of trauma and PTSD risk and to greater advances in the prevention and treatment of PTSD.

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Chapter 4 Epigenome-Wide Association of PTSD From Heterogeneous Cohorts With a Common Multi-Site Analysis Pipeline

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Abstract

Compelling evidence suggests that epigenetic mechanisms such as DNA methylation play a role in stress regulation and in the etiology of stress-related disorders such as Post-Traumatic Stress Disorder (PTSD). Here we describe the purpose and methods of an international consortium that was developed to promote collaborative studies on the role of epigenetics in PTSD. Inspired by the approach used in the Psychiatric Genomics Consortium, we brought together investigators representing seven cohorts (total N=1147) that included detailed information on trauma exposure, PTSD symptomatology, and genome-wide DNA methylation. The objective of this consortium is to increase the analytical sample size by pooling data and combining expertise in order to identify the DNA methylation patterns associated with PTSD. Several quality control and analytical pipelines were evaluated for their control of genomic inflation and technical artifacts, and a joint analysis procedure was established to derive comparable data over the cohorts for meta-analysis. We propose ways to deal with ancestry population stratification and type I error inflation and discuss the advantages and disadvantages of applying robust error estimates. To evaluate our pipeline, we report results from an epigenome-wide association study (EWAS) of age, which is a well-characterized phenotype with known epigenetic associations. Overall, while EWAS are highly complex and subject to similar challenges as genome-wide association studies (GWAS), we demonstrate that an epigenetic meta-analysis can be well-powered to identify epigenetic associations. This study can be used as a framework for consortium efforts for EWAS.

Keywords

EWAS, meta-analysis, trauma

INTRODUCTION

Traumatic events are reported by over 70% of individuals during their lifetime (Benjet et al., 2016). They have been associated with a number of deleterious outcomes, including posttraumatic stress disorder (PTSD), a psychiatric disorder characterized by intrusions, avoidance, negative alterations in thoughts and mood, and alterations in physiological arousal and reactivity (American Psychiatric Association, 2000). PTSD can be severe and disabling and is often associated with a range of comorbid psychiatric conditions, such as depression and substance use disorders (Brady, Killeen, Brewerton, & Lucerini, 2000; Najt, Fusar-Poli, & Brambilla, 2011). PTSD has also been associated with a 2.8-fold increase in suicidal thoughts and behaviors (Sareen, Houlihan, Cox, & Asmundson, 2005) as well as with a number of chronic medical conditions (Boscarino, 2008; Coughlin, 2011; David, Woodward, Esquenazi, & Mellman, 2014; Heppner et al., 2009; Jakovljevic et al., 2008; Kubzansky, Koenen, Jones, & Eaton, 2009; Kubzansky, Koenen, Spiro, Vokonas, & Sparrow, 2007). While the risk of developing PTSD depends, in part, on the nature of the traumatic event (Kessler, 2000), only a minority of those exposed to trauma develop PTSD. As a result, despite the high prevalence of lifetime trauma, the overall lifetime prevalence of PTSD in the United States is 6.8%, (Breslau et al., 1998; Kessler et al., 2005; Resnick, Kilpatrick, Dansky, Saunders, & Best, 1993), which suggests there are individual differences in resilience that, if better understood, might inform the development of new approaches to prevention and treatment.

Genetic epidemiological studies suggest that both genetic and environmental factors contribute to PTSD risk. Twin studies estimate the heritability of PTSD to be between 30-70% (Sartor et al., 2012; Sartor et al., 2011; Stein, Jang, Taylor, Vernon, & Livesley, 2002; True et al., 1993; Xian et al., 2000), with the remaining variance being attributed to environmental factors. Genetic research, both candidate gene and genome-wide association studies (GWAS), provided support for the role of genetics in the development and severity of PTSD and have begun to identify variants that account for some of the genetic influence on PTSD. The genetic loci identified in the extant GWAS have been implicated in a variety of processes, including neuroprotection, actin polymerization, neuronal function, and immune function (Almli et al., 2014; Guffanti et al., 2013; Logue et al., 2013; Xie et al., 2013). Although promising, no robust genetic variants associated with PTSD have been identified, and much work remains to be done to understand the biological basis of PTSD risk (Logue et al., 2015).

A growing body of work has explored the role of environmental influences on an individual's response to trauma. Clarifying the ways in which environmental influences might affect biological function is critical to understanding the etiology of PTSD. In this regard, epigenetic mechanisms, which can mediate environmental influences on gene function, are particularly relevant. Epigenetic modifications, such as DNA methylation at cytosine-guanine dinucleotides (CpG sites), induce changes in gene expression through structural alterations of DNA that are maintained through each round of cell division; they respond to changes in the environment, are potentially reversible, and can be targeted for disease therapies (Feinberg,

2007). DNA methylation regulates gene expression by influencing the recruitment and binding of regulatory proteins to DNA. Typically, higher methylation at gene promoter regions correlates with decreased expression of that gene, while intragenic methylation can regulate alternative promoters and enhancers (Bonasio, Tu, & Reinberg, 2010; Maunakea et al., 2010).

For example, animal studies have demonstrated that epigenetic changes—particularly alterations in DNA methylation in response to nurturing—are related to altered responses to stress (Jirtle & Skinner, 2007; Weaver et al., 2004). Similar alterations have been reported in the human literature, in both central and peripheral tissues (McGowan et al., 2009; Tyrka, Price, Marsit, Walters, & Carpenter, 2012). Considering the influence of traumatic stress on DNA methylation seen in some studies (Vinkers et al., 2015), epigenetic-based investigations may extend research findings. For example, research reporting an association of PTSD with a genetic variant in the PAC1 receptor (*ADCYAP1R1*; rs2267735) went beyond this finding to observe that PTSD severity was also correlated with methylation levels of the gene (Ressler et al., 2011). Other work has suggested an interactive effect between traumatic burden and DNA methylation in the serotonin transporter locus (*SLC6A4*) on PTSD risk, independent of the widely studied length polymorphism at this same locus (Koenen et al., 2011) and a potentially interacting effect of genetic and epigenetic variation of the dopamine receptor (*SLC6A3*) on PTSD risk (Chang et al., 2012). Indeed, stress exposure itself has been shown to alter epigenetic patterns in both animal and human studies (Moser et al., 2015; Roth, Lubin, Funk, & Sweatt, 2009; Sipahi et al., 2014). In addition to candidate gene methylation studies, a small number of studies have examined genome-wide methylation patterns in PTSD (Mehta et al., 2013; Smith et al., 2011; Uddin et al., 2010). In these first genome-wide studies of DNA methylation, immune dysregulation figured prominently among the biological networks associated with PTSD and, at a CpG site level, DNA methylation levels in several CpGs showed suggestive evidence of replication between these studies (Mehta et al., 2013; Smith et al., 2011; Uddin et al., 2010).

Although the emerging literature on epigenetic influences on PTSD is promising, the majority of research to date has been conducted with modest sample sizes, with inherent limited statistical power. Furthermore, studies of DNA methylation have been hampered by technical issues including batch effects (Harper, Peters, & Gamble, 2013) and blood cell composition (Houseman et al., 2012). Experience from large-scale genetic studies, such as the Psychiatric Genomics Consortium (PGC), underscores how large collaborative endeavors can provide the adequate sample sizes and the sufficient statistical power necessary to produce significant and replicable results. Based on this experience, the PGC-PTSD formed the epigenome-wide association study (EWAS) working group to organize an expansive collection of samples with genome-wide methylation data available for joint analyses.

Although there are many advantages to this collaborative approach, there are also challenges. First, assessments of trauma-exposure and PTSD symptoms/diagnosis differ among the cohorts, requiring harmonization of the phenotypic data. The larger PGC-PTSD has made substantial progress harmonizing phenotypes across studies, and a similar approach will be

adopted by the PGC-PTSD EWAS group in its analyses. A second challenge of consortia is to address complications in the data-sharing that is a prerequisite to increasing the power and reproducibility of the study. The trend in psychiatric genetics has been to establish large-scale consortia for the purpose of expanding sample sizes beyond what is possible based on the data from any one group. Almost universally used in current large-scale genomic consortia is a meta-analysis, through which the data from individual sites are analyzed separately and combined, based on summary statistics. In many cases, meta-analysis of individual-level results yields results comparable to those of a “mega-analysis” of pooled data from different studies (Lin & Zeng, 2010; Mathew & Nordstrom, 1999; Olkin & Sampson, 1998). However, there have been important distinctions in the way the meta-analysis strategy has been implemented across consortia, including variations in the degree to which the cleaning and analysis of the individual-level data occurs (i.e., centralized versus distributed). The cleaning and analysis of individual data in a centralized manner allows a high degree of control over the quality control (QC) process and an ability to quickly perform follow-up analyses, but poses the difficulties of requiring a larger degree of computation and storage at the consortium level and permission from all groups to share data.

The PGC-PTSD uses many of the same protocols and tools developed by the PGC (Logue et al., 2015), with the difference that not all data are centrally stored and managed, since some constituent samples that originate from military, Veteran (VA) or foreign countries are subject to additional regulatory oversight, which do not allow the sharing of individual-level genomic data. To accommodate participation for these studies, the PGC-PTSD EWAS group follows a strategy similar to that of the ENIGMA consortium (Thompson et al., 2014), in which a set of protocols and scripts are created to implement standardized QC and analysis pipelines for the Illumina HumanMethylation450 BeadChip. These scripts are performed at each participating site, and analysis results are submitted to the consortium where they are assessed, collated, and meta-analyzed. In this study, we compare the performance of two QC and two analytical pipelines to control for genomic inflation, present the final PGC-PTSD EWAS pipeline, and assess the performance of the PGC-PTSD EWAS pipeline in a meta-analysis of age.

MATERIALS AND METHODS

The PGC EWAS Cohorts

The participating cohorts, presented in Table 1, consisted of four military cohorts (MRS, PRISMO, VA-M, and VA-NCPTSD) and three civilian cohorts (DNHS, GTP, and WTC) that all measured DNA methylation (DNAm) with the Illumina HumanMethylation450K BeadChip. Descriptions of the cohorts are in the supplemental information. Each cohort consisted of PTSD cases as well as trauma-exposed controls. A total of 1,147 subjects (~50% cases) were selected for inclusion in the EWAS and were subjected to the quality control and analytical pipelines.

Posttraumatic Stress Disorder

Similar to other analyses conducted by the PGC-PTSD, our analysis required consistently defining and harmonizing PTSD diagnoses across cohorts that used different instruments and methods of diagnosis (Logue et al., 2015). We used a diagnosis of current PTSD based on the diagnostic criteria defined by each cohort's principal investigator (see supplemental information). Individuals with lifetime diagnoses of PTSD but not current PTSD were excluded from analysis.

Quality Control Procedures

We tested two quality control protocols: the eventually proposed PGC pipeline and a Functional Normalization (Funnorm) pipeline. In the PGC pipeline (Supplemental Figure 1), study investigators first conducted a visual inspection of control-probes designed to report each step of the Infinium protocol such as bisulfite conversion and hybridization efficiency. In addition, samples with probe detection call rates <90% and those with an average intensity value of either <50% of the experiment-wide sample mean or <2,000 arbitrary units (AU) were excluded. Probes with detection p-values >0.001 or that were based on less than three beads were set to missing as were probes that cross-hybridized between autosomes and sex chromosomes (Teschendorff et al., 2013). CpG sites with missing data for >10% of samples within cohorts were excluded from analysis. Probes containing single nucleotide polymorphisms (SNPs, based on 1000 genomes) within 10 base pairs of the target CpG were maintained in each dataset, but flagged and tracked throughout the analysis pipeline. This decision was based on the growing recognition that sequence variants can influence DNA methylation patterns throughout the genome. Considering our aim to identify genes important for PTSD, even if an associated CpG site is influenced by genetics, such as the *SKA2*, maintaining such probes is informative (M. P. Boks et al., 2016; Gibbs et al., 2010; Guintivano et al., 2014; Heyn et al., 2013; Rice et al., 2008; Smith et al., 2014). Normalization of probe distribution and background differences between Type I and Type II probes was conducted using Beta Mixture Quantile Normalization (BMIQ) (Teschendorff et al., 2013) after background correction. We chose BMIQ after comparing distributions of BMIQ normalized Type II probes in the Detroit Neighborhood Health Study with the raw distributions and distributions after applying the DASEN procedure in the R package watermelon (Supplemental Figure 2) (Pidsley et al., 2013).

Following normalization, batch effect removal, as implemented in the ComBat procedure of the SVA package in bioconductor, was used to account for sources of technical variations including batch and positional effects, which can cause spurious associations (Johnson, Li, & Rabinovic, 2007). Individual cohorts also controlled for additional covariates that may not have been balanced within chips but that were of interest in downstream analyses, such as case designation and sex (if relevant). Following completion of this QC pipeline, each cohort confirmed that there were no remaining sources of technical variation by examining the association of PCs of the methylation levels with chip and position using multivariate linear regression, bar plots, and heat maps.

The second QC protocol used the functional normalization procedure implemented in the R package minfi, which has been reported to remove technical variation more effectively than ComBat or other supervised methods (Aryee et al., 2014; Fortin et al., 2014). Briefly, raw IDATs were loaded into R and functional normalization applied using the default of two principal components as covariates. The resulting normalized beta matrix was then extracted and used for analysis.

Cellular Heterogeneity

DNA methylation is known to vary by cell type, which impacts the measured whole blood methylation as a result of the amalgamation of the cell type proportions in each individual sample. To control for possible confounding by individuals' underlying cell type heterogeneity, proportions of CD8, CD4, NK, B cells, monocytes and granulocytes were estimated using each individual's DNA methylation data, publicly available reference data (GSE36069), and the method described by Jaffe and colleagues (Jaffe & Irizarry, 2014; Reinius et al., 2012), based on the Houseman algorithm (Houseman et al., 2012). CD8, CD4, NK, B cell, and monocyte cell proportions were included as covariates in our statistical analyses.

Ancestry

Accounting for population stratification has become routine practice for genetic association studies, and most recently has also been shown to be of importance in DNA methylation studies (Barfield et al., 2014; Nielsen et al., 2010). GWAS methods such as principal components (PCs) derived from single-nucleotide polymorphisms (SNPs) can be incorporated into EWAS, but were not always available for all cohorts, or all samples within a cohort. An alternative based on methylation probes that proxy nearby SNPs was developed by Barfield et al. for use in European and African American subjects (Barfield et al., 2014). Here we evaluated and extended this approach to other ancestral populations as part of the PGC-PTSD EWAS pipeline.

A subset of ancestry-diverse subjects (N=128, including European Americans, African Americans, Latinos/Native Americans and 'others' including East Asians) from the Marine Resiliency study (MRS) was selected based on available genome-wide genotype data (Illumina HumanOmniExpressExome array) and matching Illumina 450K methylation data (C M Nievergelt et al., 2015). Ancestry using GWAS data was inferred as described in Nievergelt et al. (C. M. Nievergelt et al., 2013). In brief, genotypes of 1783 ancestry-informative markers (AIMs) were used to determine a subject's ancestry at the continental level using STRUCTUREv2.3.2.1, including prior population information of the HGDP reference set (Falush, Stephens, & Pritchard, 2003; Li et al., 2008). Based on these ancestry estimates, subjects were placed into one of 4 groups: European Americans, African Americans, Latinos/Native Americans and 'Others'. Principal components (PCs) were derived using Eigenstrat (Price et al., 2006).

Ancestry estimates using methylation data were derived using subsets of methylation probes in close proximity to SNPs identified by Barfield et al. (Barfield et al., 2014). Probe sets with 0bp distance (N=7,703 CpG probes), within 1bp distance (N=17,995 CpG probes), and within

10bp distance (N=50,319 CpG probes) were compared. GWAS-derived PCs were visually compared to methylation-probe derived PCs, and genotypes of SNPs in proximity of CpG sites were compared with respective CpG methylation values using Pearson correlation (r).

Statistical Analysis

Within each cohort, logit transformed β values (M-values) (Du et al., 2010) were modeled by linear regression as a function of PTSD, adjusting for sex, age, the estimated cell proportions, and ancestry using the either the first three PCs from GWAS data if available or the second through fourth PCs from EWAS data. QQ-plots of the PTSD p-values were examined for evidence of genomic inflation due to unaccounted technical variation or other confounders. In addition, the genomic inflation factors (λ) were calculated for each study. Two adjustments were considered to improve the precision of the estimated variances. First, moderated t-statistics were calculated using the empirical Bayes method implemented in the R package limma (Smyth, 2005). Second, HC3 robust standard errors, which have been shown to be the most effective in samples smaller than 250, were calculated using the R package sandwich (Long & Ervin, 2000; Zeileis, 2004).

Cohort-level analysis results were combined using the inverse normal method (Marot, Foulley, Mayer, & Jaffrézic, 2009). Briefly, one-sided p-values for each CpG site in each study were calculated from the t-statistics. Next, z-scores were calculated from the one-sided p-values and weighted by the number of subjects in each study relative to the total in the meta-analysis. Two-sided p-values of the z-score were then calculated, and genomic inflation examined. Finally, p-values were adjusted for multiple-testing using the False Discovery Rate (FDR) procedure at the Type I error rate level of 5 percent (Benjamini & Hochberg, 1995).

Sensitivity Analysis

We also conducted a sensitivity analysis by modeling methylation as a function of age along with covariates for gender and ancestry and meta-analyzed as outlined above except without using an empirical Bayes adjustment to simplify our analysis in order to help identify statistical issues that may cause inflation or deflation of our results. Numerous robust associations between age and DNAm have been reported, and we examined whether the increased sample size in the meta-analysis increased the number of significant CpG sites over the individual studies (Bocklandt et al., 2011; Hannum et al., 2013; Horvath, 2013; Horvath et al., 2012; Weidner et al., 2014). In addition, we measured concordance between studies by estimating the correlation between the t-statistics of the age variable calculated without either standard error adjustment.

Power

Power for EWAS is more favorable compared to GWAS as a result of the continuous nature of the DNA methylation measures, but instead suffers from poor distribution including low variances and heteroscedasticity. The power to detect a differentially methylated CpG site

depends on the percent difference in methylation between cases and controls, the pooled variation in methylation (σ) across CpG sites, and the number of cases and controls (Liu & Hwang, 2007). We conducted simulations to test the smallest mean difference between PTSD cases and controls we could detect based on our projected sample size and a $\sigma=0.43$, which represents the highest variation reported in one of our cohorts, and thus a very conservative estimate (Orr & Liu, 2009).

RESULTS

Participating Cohorts

Sample characteristics for studies that have contributed data to this first PGC-PTSD EWAS study are listed in Table 1 (N=1147). Three of the seven studies are composed of civilians, while the remaining studies include active duty and veteran military populations. The majority of participants, especially from the military cohorts, were male (73%) and of European American (EA) descent (56%). All participants were exposed to trauma, and half suffered from PTSD (50%). Data collection occurred across the United States (e.g., Atlanta, Detroit, San Diego, Durham, Boston) and Europe. While a few studies used clinical interviews, the majority of studies used self-report ratings of PTSD symptoms. A detailed description of participating cohorts is provided in the supplementary information.

Power

The power analysis shows that with our sample of 573 cases and 574 controls (N=1147), we are sufficiently powered to find at least one CpG site with a mean methylation difference of 0.08 between cases and controls (Figure 1).

Ancestry

We investigated the utility of DNA methylation-based ancestry estimates based on CpGs with nearby genetic variants in *cis* as proposed by Barfield et al. (Barfield et al., 2014). A comparison of CpG probes with SNPs within 1bp distance (N= 17,995) and CpG probes with SNPs within 10bp distance (N= 50,319) showed a higher genotype-methylation correlation for the 1bp probes ($r=0.29$, $p=1.8 \times 10^{-15}$) than the 10bp probes ($r=0.06$, $p=0.0015$). Figure 2 shows a SNP-derived PC plot based on available GWAS data including PC1 and PC2 (panel A), PCs 2 and 3 from the methylation-based CpGs with a SNP 1 bp from the probes (panel B) and the PCs from CpG probes within 10bp of a SNP (panel C). Supplemental Figure S5 shows PCs 2, 3, 4, and PCs 2, 3, and 6 for, respectively, CpGs within 1 bp and 10 bp of a SNP. These results along with the genotype-methylation correlations show that the use of DNA methylation ancestry estimates (PCs 2–4) using probes within 1 bp of a SNP provide reliable results and are suitable as ancestry covariates in our analyses.

Quality Control Results

The number of samples and probes, not including cross-reactive probes, removed in our proposed PGC-EWAS pipeline ranged from 677 to 10,218 across studies (Supplemental Table S3). Figure 3 presents the genomic inflation factors from the analysis of PTSD for each individual study using two different quality control methods as well as two different analysis pipelines: (1) our proposed PGC-EWAS pipeline, described in detail in the Methods section above, as well as (2) the Functional Normalization (Funnorm) QC pipeline. Resulting data from each QC pipeline were then subjected to linear regression analysis, performed with empirical Bayes and HC3 standard errors, respectively. In studies using the Funnorm pipeline there were large variations in the genomic inflation factor with two studies showing high inflation (DNHS, GTP) and two studies showing substantial deflation (MRS, VA-NCP) regardless of whether empirical Bayes or HC3 standard errors were used. Using the PGC-EWAS pipeline and HC3 standard errors, six of the seven studies showed genomic deflation with $\lambda < 1.0$, while only one study was deflated when using empirical Bayes standard errors (Supplemental Table S1). These results indicate that the PTSD-PGC EWAS pipeline, combined with empirical Bayes standard errors, is the preferred method for cohorts participating in our meta-analysis.

Sensitivity Analysis: Age Meta-Analysis Results

Results for the age analysis using our pipeline and no standard error adjustment are presented in Table 3. All studies reported numerous FDR-significant CpG sites but substantially more significant results were reported for the combined meta-analysis. MRS and PRISMO reported the fewest significant sites. However, the age range for participants in these studies was narrower as both studies recruited active military personnel. The correlations of the t-statistics ranged from 0.171 to 0.692 when all sites were analyzed and from 0.441 to 0.886 among the FDR significant sites (Supplemental Figure 3). The strong correlations of the most significant sites indicate that each cohort retained the biological signal of age after QC. Of the 494 CpG sites reported to have been associated with age, 326 were significant after FDR-correction (Supplemental Table 2). In addition, a forest plot of the most significant CpG site, representative of the FDR significant sites, shows a consistent direction of effect in each study (Supplemental Figure 4).

DISCUSSION

PTSD is unique among psychiatric disorders in that its diagnosis requires exposure to a significant traumatic event. With an environmental exposure embedded into the etiology of the disorder, the PTSD diagnosis affords an unusual opportunity to identify individual differences in the biological response to trauma to increase risk for, or resilience to, the disorder. Here we have introduced an international collaboration that has been established to identify epigenetic associations--specifically, DNA methylation--that are related to risk for, or resilience to, PTSD. We presented the development of a consistent pipeline for processing and quality control of epigenome-wide association data comparing two quality control approaches and statistical

pipelines. In our analysis of PTSD, we found that our proposed PGC-EWAS pipeline controlled for genomic inflation and deflation more consistently than functional normalization, regardless of the standard error correction used. In light of these findings, we encourage consortia to implement our quality control pipeline including checks for genomic inflation and strengthened associations with age before meta-analyzing across studies.

Through these collaborative efforts to analyze existing DNA methylation data from blood obtained from both military and civilian cohorts, we are poised to collectively address one of the main challenges of psychiatric genomics, namely the need for large, harmonized samples to adequately power genome-scale analyses. The current collaborative dataset allows detection of methylation differences around seven percent, larger than most reported methylation differences (Vinkers et al., 2015). Additional EWAS datasets that are forthcoming will likely prove essential to detecting PTSD-associated DNA methylation differences in our planned meta-analyses. Moving forward, we anticipate that our collaborative efforts will grow to include additional cohorts from around the world; indeed, in the last year alone, several new studies have expressed interest in participating in future EWAS analyses as their data become available. In addition to the DNA methylation analysis, the close allegiance with the PGC-PTSD group has laid the foundation for integrating data from genome-wide association (GWAS), EWAS, and gene expression/transcriptome analyses. In combination with other biological measures and coordinated neuroimaging efforts (Logue et al., 2015) that may become accessible through this collaboration, these system-wide integrations will facilitate a more complete understanding of the molecular architecture and biological underpinnings of PTSD.

The harmonization of some study characteristics paired with the demographic and clinical diversity of the samples, including the differences between military and civilian trauma, allows us the opportunity to identify DNA methylation patterns predictive for specific groups of individuals and types of trauma. This will not only provide insight into the heterogeneity of PTSD, but may also help explain mechanisms for the variation in conditional effects of different types of trauma on PTSD (Wisco et al., 2014). Additionally, it will also provide a framework from which DNA methylation may be informative for early prediction and treatment.

Looking ahead, we are optimistic that our PTSD EWAS collaboration will identify blood-based DNA methylation signatures that associate reliably with PTSD. Identification of robust peripheral biomarkers is an important first step and has potential for early detection and prevention. The ultimate goal is to provide new insights into the etiology of PTSD. To truly understand the mechanistic basis of PTSD, it will be critical to compare our blood-derived epigenetic biomarkers with those from other tissues, in particular brain tissue. As a first pass, DNA methylation-based biomarkers that associate with PTSD at particular CpG sites in blood can, at this time, be compared to CpG site derived from brain tissues, thanks to the Epigenomic Roadmap datasets (Bernstein et al., 2010; Kundaje et al., 2015). However, these comparisons will be limited to a comparison of DNA methylation levels in brain and blood in general, as the current data are not representative of PTSD. Over time, however, the development of PTSD brain

biobanks of brain and other tissue types, including blood cells, will help us to pinpoint whether blood-derived, DNA methylation biomarkers of PTSD reflect similar alterations in brain tissue. Recent work suggests that this is possible for certain pathways (Daskalakis, Cohen, Cai, Buxbaum, & Yehuda, 2014). Collectively, these cross-tissue efforts will provide insight into the biological pathways underlying PTSD vulnerability and will ultimately facilitate new treatment and prevention.

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Conflict of Interest

Wiley-Blackwell requires that all authors disclose any potential sources of conflict of interest. Any interest or relationship, financial or otherwise, that might be perceived as influencing an author's objectivity is considered a potential source of conflict of interest. These must be disclosed when directly relevant or indirectly related to the work that the authors describe in their manuscript. Potential sources of conflict of interest include but are not limited to patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. The existence of a conflict of interest does not preclude publication in this journal.

If the authors have no conflict of interest to declare, they must also state this.

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Table 1: PGC-PTSD EWAS Cohorts

		Civilian			Military			
	Total	DNHS	GTP	WTC	MRS	PRISMO	VA-M	VA-NCP
N	1147	100	270	180	126	62	176	233
Mean Age (SD)	38.0 (-)	53.6 (14.0)	41.7 (12.4)	49.7 (8.3)	22.2 (3.0)	27.1 (9.2)	34.9 (9.9)	32.0 (8.4)
Current PTSD, %	50%	40%	39%	47%	50%	50%	49%	69%
Male, %	73%	38%	30%	100%	100%	100%	78%	90%
Race, %								
White	56%	15%	5%	76%	57%	100%	100%	74%
Hispanic	6%	0%	0%	0%	25%	0%	0%	14%
Black	33%	85%	94%	4%	8%	0%	0%	9%
Asian	1%	0%	0%	0%	3%	0%	0%	2%
Other	3%	0%	0%	20%	0%	0%	0%	0%

Table 2: Age Results using PGC QC and Analysis pipeline

Study	Sites	Sites with (FDR < 0.05)	Sites with ($p < 5 \times 10^{-5}$)	Sites with ($p < 5 \times 10^{-6}$)	Sites with ($p < 5 \times 10^{-7}$)
DNHS	455,079	4,766	1,744	678	299
GTP	453,351	59,100	21,562	14,299	9,586
MRS	455,601	210	311	99	34
PRISMO	446,688	246	316	121	41
VA-M	455,641	42,474	12,913	7,213	4,159
VA-NCPTSD	453,747	35,217	10,522	6,331	3,991
WTC	455,340	14,239	5,013	2,730	1,525
Meta-Analysis	444,164	119,308	57,332	46,629	38,656

Figures

Figure 1: Sample size vs. power to detect differentially methylated CpG sites. The black curve indicates the number of cases and controls necessary to find a differentially methylated if only one CpG site exists, while the grey line indicates the size necessary if 10 differentially methylated sites exist.

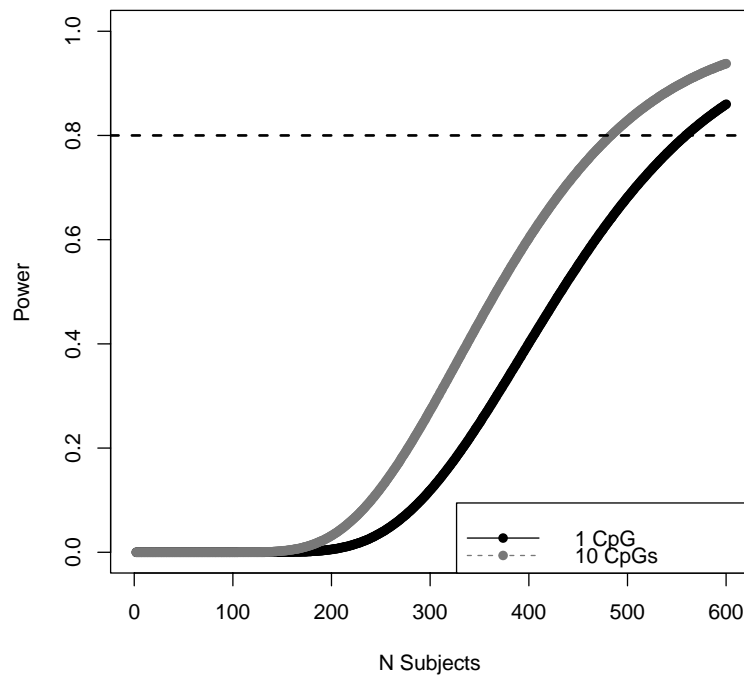


Figure 2: PTSD genomic inflation factors (λ) by quality control pipeline (PGC vs. Funnorm) and standard error adjustment method (empirical Bayes vs. HC3).

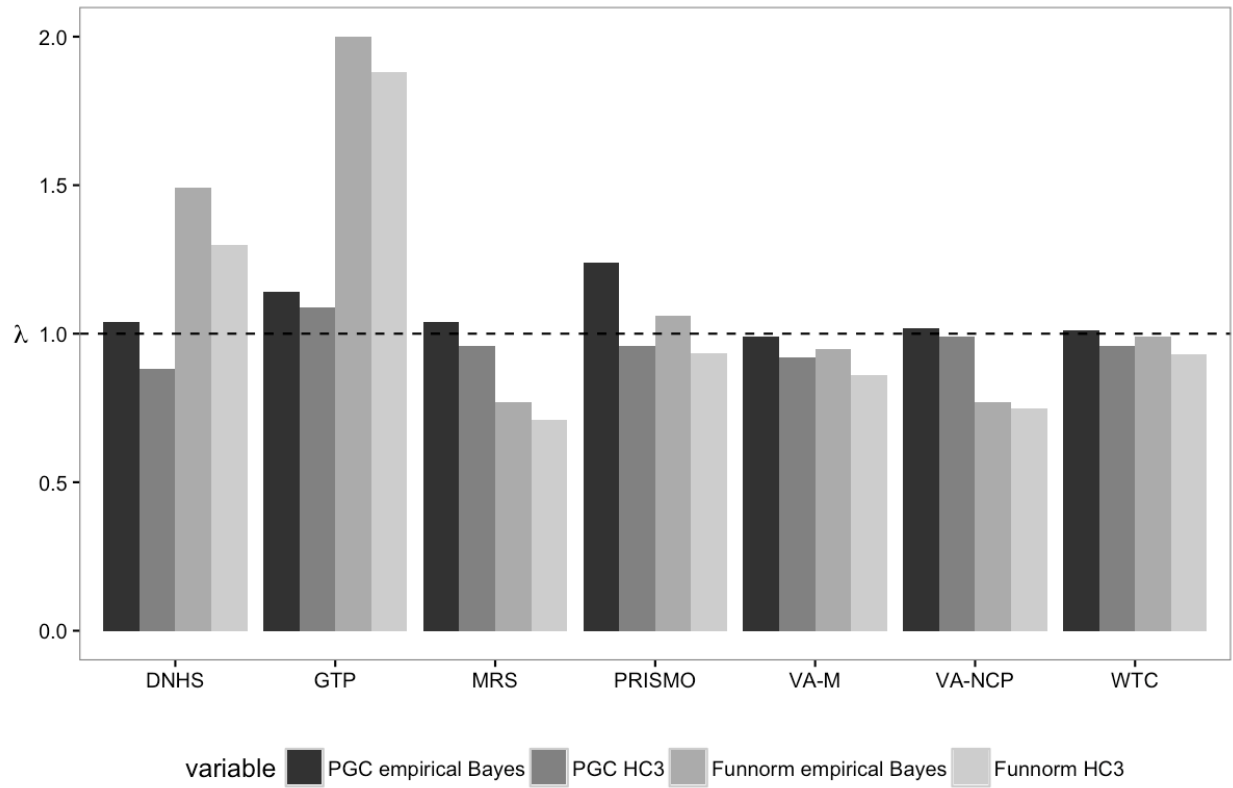
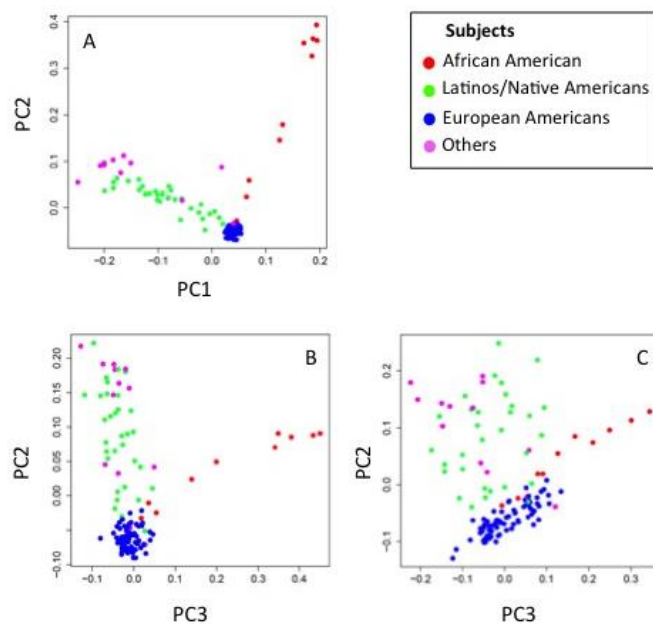


Figure 3: Ancestry inference using SNPs vs. methylation probes in 128 participants of the Marine Resiliency Study (MRS). (A) Principal component (PC) plot showing ancestry inferred using SNPs from a genome-wide association study (GWAS). PC plots based on CpG probes with SNPs within 1bp distance (B) and with SNPs within 10bp distance (C), respectively. Subject are placed into 4 ancestral groups based on ancestry estimates using ancestry-informative SNPs and a reference panel (see methods).

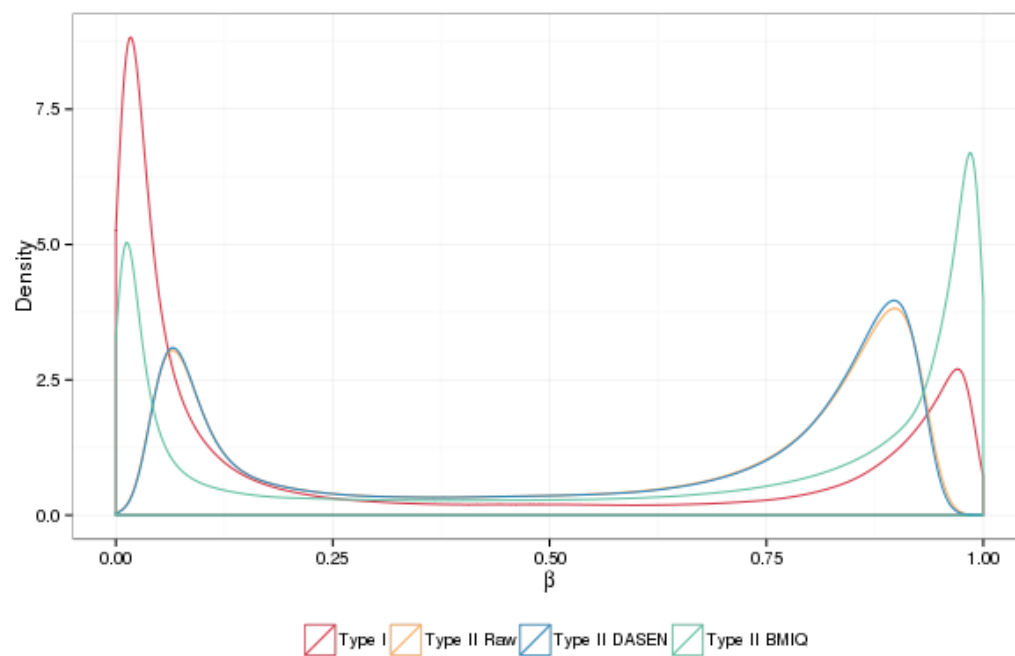


Supplemental Information

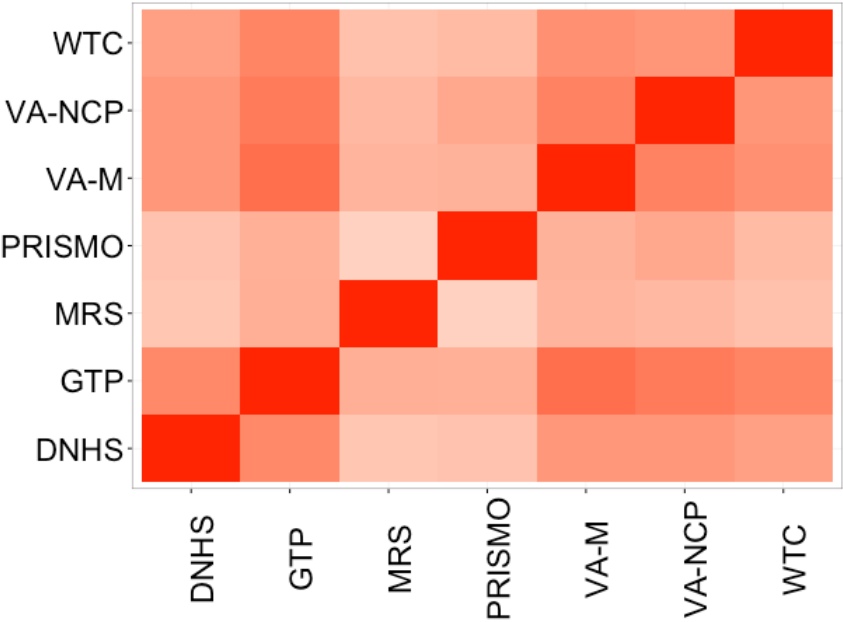
Supplemental Table 1: PTSD Genomic Inflation by Quality Control Pipeline

Study	PGC Sites	N	PGC empirical Bayes	PGC Pipeline HC3	Funnorm N Sites	Funnorm empirical Bayes	Funnorm HC3
DNHS	455,079		1.04	0.88	485,512	1.49	1.30
GTP	453,351		1.14	1.09	485,512	2.00	1.88
MRS	455,601		1.04	0.96	485,512	0.77	0.71
PRISMO	446,688		1.24	0.96	485,512	1.06	0.93
VA-M	455,641		0.99	0.92	485,512	0.95	0.86
VA-NCP	453,747		1.02	0.99	485,512	0.77	0.75
WTC	455,340		1.01	0.96	485,512	0.99	0.93

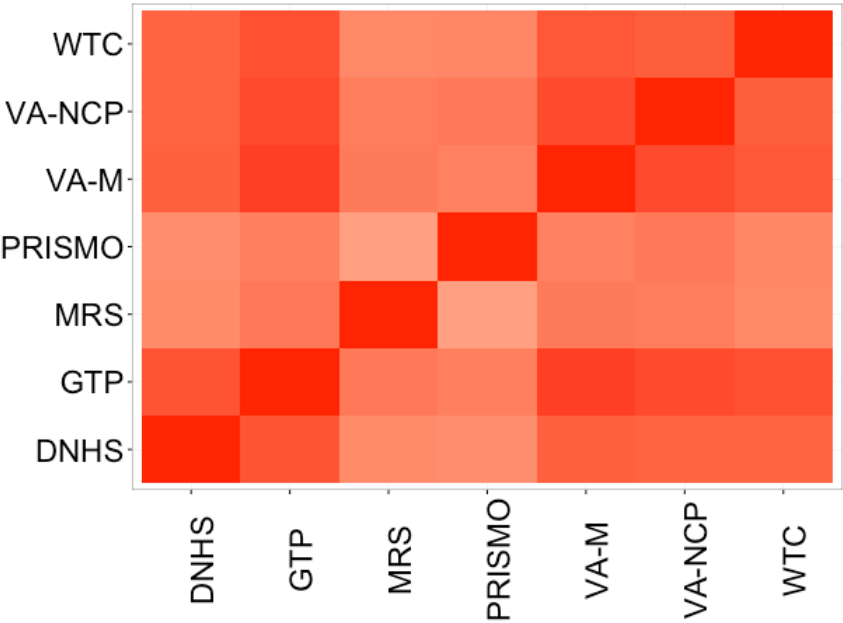
Supplemental Figure 2: Comparison of Type-II probe normalizations in the Detroit Neighborhood Health Study



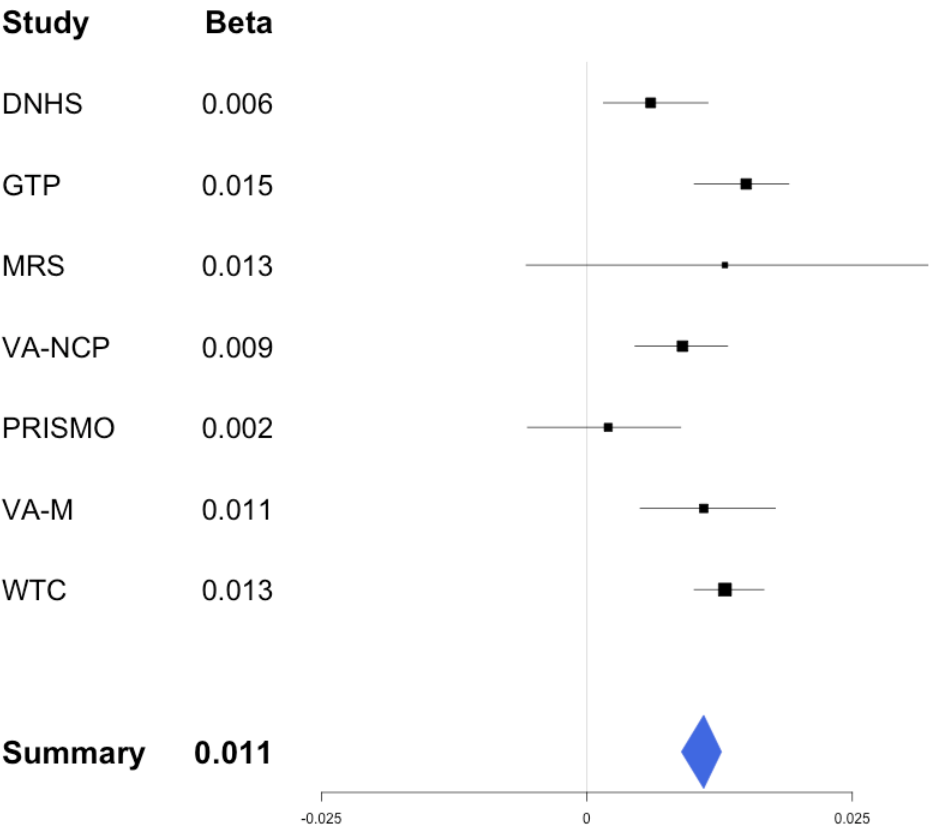
Supplemental Figure 3A: Age analysis t-statistic correlations across all CpG sites.



Supplemental Figure 3B: Age analysis t-statistic correlations for the FDR Significant Sites.



Supplemental Figure 4: Forest Plot of the top CpG site from the analysis of age. Forest plots for significant sites show common direction of effect



PGC EWAS Cohorts

Detroit Neighborhood Health Study

See reference for details (Uddin et al., 2010). Briefly, participants (N=1547 at the baseline wave) were assessed for PTSD symptoms using the PTSD checklist (PCL-C), a 17-item self-report measure of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) symptoms, and additional questions about duration, timing, and impairment or disability due to the symptoms (Blanchard, Jones-Alexander, Buckley, & Forneris, 1996). Participants were initially asked to identify potentially traumatic events (PTEs) that they experienced in the past from a list of 19 events. PTSD symptoms were then assessed by referencing two traumatic events that the respondent may have experienced: one that the participant regarded as the worst and one randomly selected event from the remaining PTEs a respondent may have experienced. Respondents were considered affected by lifetime PTSD if all six DSM-IV criteria were met in reference to either the worst or the random event. Current PTSD is defined as meeting the criteria for lifetime PTSD, with symptoms reported during the past month. The DNHS was approved by the institutional review board at the University of Michigan and University of North Carolina at Chapel Hill.

Grady Trauma Project

See reference for details (Binder et al., 2008). The modified PTSD Symptom Scale (PSS), a psychometrically valid 17-item self-report scale assessing PTSD symptomatology over the prior 2 weeks, was used to assess PTSD. Consistent with prior literature, the PSS frequency items (0 indicates not at all to 3 indicates ≥ 5 times a week) to obtain a continuous measure of PTSD symptom severity ranging from 0 to 51. For this sample, the PSS frequency items had standardized $\alpha=.90$ (mean [SD], 13.81 [11.96]). No clearly established PSS cutoff score for PTSD diagnosis has been established; however, DSM-IV criteria for PTSD can be applied to PSS frequency items to create a proxy variable for PTSD diagnostic status. Demographic variables including age, sex and race were assessed through self-report. The Institutional Review Boards of Emory University School of Medicine and Grady Memorial Hospital approved this study.

Marine Resiliency Study

See reference for details (Baker et al., 2012; C M Nievergelt et al., 2015). PTSD was diagnosed up to 3 times, once before deployment and 3 and/or 6-month post-deployment. Post-traumatic stress (PTS) symptoms were assessed using a structured diagnostic interview, the Clinician Administered PTSD Scale (CAPS), and PTSD diagnosis followed the DSM-IV criteria for partial and full PTSD. The study was approved by the University of California – San Diego Institutional Review Board.

Mid-Atlantic Mental Illness Research Education and Clinical Center PTSD Study

See reference for details (Ashley-Koch et al., 2015). PTSD was diagnosed using the Structured Clinical Interview for DSM-IV Disorders (SCID) administered by trained interviewers. In accordance with the DSM-IV, PTSD consists of three symptom clusters. These include re-experiencing symptoms (B symptoms), avoidance and numbing symptoms (C symptoms) and hyperarousal symptoms (D symptoms). Total PTSD symptoms and symptom clusters (B, C, or D) were measured using the Davidson Trauma Scale for all veterans including individuals with current PTSD diagnosis and controls. The research was reviewed and approved by the Institutional Review Boards at the Salisbury VA, Hampton VA, Durham VA and Duke University Medical Centers.

National Center for PTSD

See reference for details (Logue et al., 2013). VA study participants were administered the CAPS, a 30-item structured diagnostic interview that assesses the frequency and severity of the 17 DSM-IV PTSD symptoms, 5 associated features and functional impairment, to assess current and lifetime PTSD symptoms. The Institutional Review Boards at two VA health care facilities approved the study.

PRISMO

All subjects in the DD were male participants in PRISMO, a large prospective study of 1,032 well-characterized Dutch military soldiers scheduled for a deployment of at least four months to Afghanistan with longitudinal follow-up. Baseline measures were recorded at one month pre-deployment. Follow-up was performed at one month and six months post-deployment, and data from the baseline and six-month follow-up were used for this analysis. A subset (total N=93) of three similarly sized subgroups of PRISMO study participants were pre-selected based on the level of traumatic stress exposure and the presence of PTSD symptoms: i) a subgroup showing high combat-trauma exposure (7.3 ± 2.9) and high levels of post-deployment PTSD symptoms (45.3 ± 8.6); ii) a subgroup showing high combat-trauma exposure (8.6 ± 2.3) and a low severity of PTSD symptoms (26.0 ± 3.7); and iii) a subgroup showing low combat-trauma exposure (0.4 ± 0.5) and low levels of post-deployment PTSD symptoms (25.1 ± 3.7).

Blood samples were collected six months after deployment. The blood cell-type composition was investigated using flow cytometry, implemented in the clinical laboratory of Utrecht University Medical Center, as previously reported (M P Boks et al., 2015). The presence and severity of symptoms of PTSD over the previous four weeks were assessed with the 22-item Self-Report

Inventory for PTSD (SRIP), which has good reliability and validity. Differences in PTSD symptoms between time points were log-transformed to improve the distribution. Exposure to traumatic stress during deployment was assessed with a 19-item deployment experiences checklist, as previously reported (van Zuiden et al., 2011).

World Trade Center 9/11 First Responders

See reference for details (Herbert et al., 2006). First responders were administered the SCID PTSD module with interval instructions (i.e., worst episode of symptoms since 9/11/2001) and SCID items were modified to assess PTSD symptoms in relation to traumatic World Trade Center exposures. The Committees on Research Involving Human Subjects at Stony Brook University approved the study.

Nagy Youssef's contributions (added for the purpose of this thesis): Nagy Youssef was one of the fellow investigators at the Mid-Atlantic MIRECC when he was at Duke University and Durham VA. He interviewed research patients, supervised SCID and PCL administration, collected the data with other MIRECC investigators, supervised research assistants, and reviewed and edited this paper.

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Chapter 5 A 3-Year Longitudinal Study Examining the Effect of Resilience on Suicidality in Veterans

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Abstract

Background: This study prospectively evaluated the correlation and role of resilience and resilience factors in predicting suicidal ideation and attempts in veterans.

Methods: In this 3-year longitudinal study, 178 Iraq and Afghanistan War veterans were evaluated for a number of clinical and demographic variables. Longitudinal follow-up was performed at approximately 3 years.

Results: Resilience at the initial assessment predicted lower suicidality at follow-up, controlling for suicidality at the initial assessment, suggesting a protective effect for resilience. With respect to specific domains of resilience, secure relationships and positive acceptance of change significantly predicted lower suicidality.

Conclusions: These findings have important implications for clinical care and for guiding future research efforts to increase resilience among returning soldiers.

Keywords

Suicidality, psychological resilience, veterans, resilience factors, secure relationships

INTRODUCTION

Studies indicate high rates of mental health problems (Erbes, Westermeyer, Engdahl, & Johnsen, 2007; Friedman, 2006; Hoge, Auchterlonie, & Milliken, 2006; Jakupcak, Luterek, Hunt, Conybeare, & McFall, 2008; Milliken, Auchterlonie, & Hoge, 2007; Zivin et al., 2007) and suicidality (Kaplan, Huguet, McFarland, & Newsom, 2007) among returning soldiers. As military troops return from Iraq and Afghanistan, addressing post-deployment adjustment problems, including suicidality, is an important priority (Hoge et al., 2006; Hoge et al., 2004; Milliken et al., 2007).

Suicide risk factors in veteran populations have been studied extensively. Studies have indicated that psychiatric disorders, particularly depression and substance use disorders, are strong risk factors for suicide in most cases (American Psychiatric Association, 2003). Other important risk factors include posttraumatic stress disorder (PTSD) (Guerra, Calhoun, & Mid-Atlantic Mental Illness Research, 2011; Panagioti, Gooding, & Tarrier, 2009), previous suicide attempts, and demographic factors such as age, sex, and ethnicity (American Psychiatric Association, 2003). Additionally, combat trauma (Bryan & Cukrowicz, 2011) and childhood abuse (Roy, 2011) have been found to be risk factors for suicide. Some of these factors are modifiable, such as reducing or controlling symptoms of psychiatric disorders, while others, such as age, sex, and history of suicide attempts, are not.

Despite extensive literature on understanding and treating risk factors for suicide, little empirical progress has been made toward preventing suicide in military service members, among whom there has been an alarming increase in suicide (Bachynski et al., 2012). In one study, the risk of death from disease in general did not differ between veterans and the nonveterans. However, veterans were twice as likely (adjusted hazard ratio [HR], 2.13; 95% CI, 1.14 to 3.99) to die of suicide compared with nonveterans in the general population (Kaplan et al., 2007).

Clinical and research data from other disciplines have indicated that reducing risk factors should be paired with boosting protective factors. For example, if eliminating risk factors for myocardial infarction (e.g., smoking cessation) is augmented with protective factors (e.g., physical exercise), marked reduction in rates of myocardial infarction can be achieved. The same approach could help reduce suicide rates.

However, limited data are available on protective factors against suicide, especially among veterans. One possible protective factor is resilience. Resilience is a construct that has received more attention in recent years. It is defined by Connor and Davidson as “qualities that enable one to thrive in the face of adversity” (Connor & Davidson, 2003). It also has been measured and defined by the well-validated Connor-Davidson Resilience Scale (CD-RISC) (Connor & Davidson, 2003; Yu, Lau, Mak, Zhang, & Lui, 2011) and has been suggested to be multidimensional (Brunet, McDonough, Hadd, Crocker, & Sabiston, 2010; Leve, Fisher, & Chamberlain, 2009; O'Donnell, Schwab-Stone, & Mueeed, 2002; Wright, Fopma-Loy, & Fischer, 2005). Resilience can improve in patients with PTSD, with significant reduction in CD-RISC scores after pharmacotherapeutic and psychotherapeutic interventions (J. Davidson et al., 2008; J. R. Davidson et al., 2005).

Studies that have examined resilience and suicidality assessed this relationship cross-sectionally. These studies suggested that resilience might be a protective factor against suicide risk (Green, Calhoun, Dennis, & Beckham, 2010; Lockwood & Youssef; Nruham, Holen, & Sund, 2010; Pietrzak et al., 2010; Roy, Carli, & Sarchiapone, 2011).

However, assessment of resilience longitudinally is needed to understand this relationship across time. Moreover, there is no study that has examined which factor(s) (of 5 factors of resilience described by the Connor and Davidson factor analysis (Connor & Davidson, 2003)) is/are correlated with suicide.

Understanding the relationship between resilience and suicide in returning veterans is helpful for many reasons. First, it could help identify individuals who are at the highest risk for suicide, therefore providing further training or excluding these individuals from situations associated with high trauma exposure. Second, interventions that have the most resilience-enhancing effect could be further examined to determine if they could lower the suicide rate. Finally, examining which factor(s) are correlated with resilience might help in psychotherapeutic and psychosocial treatment development to boost resilience and has implications for treatment policy.

This study extends our previous work (Green et al., 2010; Lockwood & Youssef). Our *a priori* hypothesis is that across time, higher resilience would predict lower suicidality. Second, we also explored how the 5 individual factors of resilience (Connor & Davidson, 2003) predicted protection against suicidality across time.

METHODS

Participants and Procedures

In this longitudinal study, 176 Operation Enduring Freedom, Operation Iraqi Freedom and Operation New Dawn (OEF/OIF/OND) veterans were interviewed at the Veterans Integrated Service Network (VISN) 6 Mental Illness Research, Education, and Clinical Center (MIRECC). The MIRECC houses a research registry of veterans who served in the US Armed Forces after September 11, 2001, and volunteered to be considered for research. All veterans were separated from active duty or were in the National Guard/Reserve.

Independent variables on veteran risk factors were gathered at baseline interviews, which occurred between 2005 and 2008. This was designated at time 1 (T1). Veterans in the MIRECC registry were recruited through mailings, advertisements, and clinician referrals. Veterans completed informed consent procedures that were approved by VA institutional review boards at multiple sites in North Carolina and Virginia and afterward were administered questions concerning post-deployment adjustment. Veterans were compensated for their time and travel expenses.

Dependent variables on veterans' suicidality were gathered from follow-up interviews, designated as time 2 (T2). An average of 3 years after the baseline interview, veterans in the MIRECC registry were contacted and asked to participate in a National Institute of Mental Health study, which served as the follow-up interview for the current study. Because this study is part

of a registry database with a 3-year follow-up, the raters did not know the outcome measures or the purpose of this analysis while evaluating participants. Veterans were compensated for their time and travel expenses after the interviews.

Measures

Beck Scale for Suicide Ideation (BSI): Suicidality was assessed using the 21-item, self-report BSI (Beck & Steer, 1991). Severity of suicidal thoughts, intent, and plans are assessed in items 1 to 19. The number of previous suicide attempts and the seriousness of the attempt to die associated with the last attempt are assessed in items 20 and 21. Item responses are rated on a 3-point scale ranging from 0 to 2 and then summed to arrive at a total score. The BSI has demonstrated strong internal reliability (reported α coefficient between 0.90 and 0.97 (Beck, Steer, & Ranieri, 1988; Steer, Rissmiller, Ranieri, & Beck, 1993)). BSI has reasonable convergent, discriminative and predictive validity (Beck, Brown, & Steer, 1997; Beck et al., 1988; Brown, Beck, Steer, & Grisham, 2000; Cochrane-Brink, Lofchy, & Sakinofsky, 2000). Brown et al. used a cutoff score of 3 to create a dichotomous index of suicidality, using survival analyses. They determined that a cutoff score of 3 on the BSI yielded the highest hazard ratio in prediction of suicide risk. Given these data, as well as the fact that the BSI deviated markedly from normality in our sample, we employed this cutoff score in our current study to create a dichotomous index (≤ 2 or ≥ 3) of suicidality.

Connor-Davidson Resilience Scale (CD-RISC):

The CD-RISC is a reliable (Cronbach α = 0.96 in the current sample) and valid (Connor & Davidson, 2003) instrument for measuring resilience. The scale was administered and validated in several different populations, including a community sample, primary care outpatients, general psychiatric outpatients, participants with generalized anxiety disorder and participants with PTSD.

Items on the CD-RISC assess resilience and include items such as “can deal with whatever comes,” “past success gives confidence for new challenge,” “see the humorous side of things,” “coping with stress strengthens,” “tend to bounce back after illness or hardship,” “things happen for a reason,” “best effort no matter what,” “not easily discouraged by failure,” “think of self as strong person,” “strong sense of purpose” and “in control of your life.”

The scale demonstrated sound psychometric properties (Connor & Davidson, 2003). The CD-RISC has demonstrated sensitivity to treatment effects with several therapies (including sertraline, paroxetine, venlafaxine, and cognitive behavioral therapy) in PTSD patients over time (J. Davidson et al., 2008; J. R. Davidson et al., 2005). This scale consists of 25 items. Each item is rated on a 5-point Likert scale with a possible score from 0 to 100. Higher scores reflect greater resilience. This was used to measure global resilience.

Factor analysis as performed by Connor and Davidson yielded 5 factors. These factors were interpreted in the following manner: factor 1 reflects personal competence, high standards, and tenacity; factor 2 relates to trust in one’s instincts, tolerance of negative affect, and strengthening effects of stress; factor 3 corresponds to the positive acceptance of change and

secure relationships; factor 4 relates to control; and factor 5 to the effect of spirituality. The 5 factors' eigenvalues were 7.47, 1.56, 1.38, 1.13, and 1.07, respectively (Connor & Davidson, 2003). In this study, we examined how the 5 factors of resilience at time 1 predicted suicidality at time 2.

Davidson Trauma Scale (DTS):

PTSD severity was assessed using the DTS (J. R. Davidson et al., 1997; McDonald, Beckham, Morey, & Calhoun, 2009). The DTS is a brief global assessment scale for PTSD symptoms. It includes 17 items corresponding to DSM-IV symptoms of PTSD. The 17 items are rated by frequency and severity. Reliability and validity of the DTS has been demonstrated in veterans who have served since September 11, 2001 (McDonald et al., 2009).

Alcohol Use Disorders Identification Test (AUDIT):

AUDIT is a 10-item, self-report screening questionnaire used to identify individuals with problematic patterns of alcohol consumption. AUDIT is divided into 3 domains: hazardous alcohol use (questions 1 to 3), dependence symptoms (questions 4 to 6), and harmful alcohol use (questions 7 to 10). Each response has a score ranging from 0 to 4. A total score of ≥ 8 in men, or ≥ 7 in women, indicates a strong likelihood of hazardous and harmful alcohol use, as well as possible alcohol dependence. The AUDIT has been validated for DSM-IV alcohol use disorders.

Analysis procedures

All statistical analyses were performed using SAS, version 9.2 for Windows (SAS Institute). Univariate analyses were used to describe the sample. Spearman correlation analyses were conducted to ascertain bivariate relationships between T2 suicidality and T1 variables. Analyses by multiple logistic regression were subsequently subjected to stepwise deletion to obtain a reduced model; exclusion criteria were set at a conservative level of $p < 0.05$. Specifically, T2 suicidality was regressed on T1 suicidality, T1 resilience, and other clinical and demographic covariates. A parallel analysis was conducted that included multiple domains of resilience derived from previous factor analyses (Connor & Davidson, 2003).

RESULTS

Demographic and clinical characteristics are shown in Table 1. Participants (N=176) were mean age of 39 (SD=10.6) and were primarily white (63%). The study sample was largely men (82%), and the majority completed post-high school education (57%).

Resilience factors protective against suicidality

Table 2 displays Spearman correlation coefficients between suicidality and resilience factors. There was a significant negative correlation between suicidality and secure relationships ($r_s = -0.34$; $p < 0.0001$) and control ($r_s = -0.29$; $P = .0004$). A trend was found between suicidality and tolerance ($r_s = -0.16$; $P = .049$). There was neither significant correlation between suicidality and tenacity, nor between suicidality and spirituality.

We also examined resilience factors (Connor & Davidson, 2003) most predictive of suicidality across time using multivariate regression. The factor corresponding to secure relationships and positive acceptance of change ($r^2 = 0.04$; $F = 8.19$, $p = 0.005$) was most predictive both in the bivariate analysis (Table 2) and in the multivariate regression (Table 3). Although control (factor 4 of resilience) was correlated with suicidality in the bivariate correlation, it was not correlated when examined in the multivariate model.

Resilience is protective against suicidality in multivariate model

The results of the multivariate forward stepwise regression are summarized in Table 4. Resilience at baseline (T1) was found to be predictive of suicidality at follow-up (T2) ($R^2 = 0.17$; $F = 3.95$; $p = 0.0485$), controlling for several demographic and clinical variables (Table 4) including suicidality at baseline ($p < 0.0001$) (Connor & Davidson, 2003). Low resilience was a stronger predictor of suicidality than alcohol misuse (on the AUDIT) and PTSD.

DISCUSSION

In this study, suicidality and resilience have been shown to be inversely related across time. Longitudinal follow-up was performed at approximately 3 years. This suggests a protective effect of resilience on suicidality. Of the 5 factors of resilience, the factor corresponding to secure relationships and positive acceptance of change was most significantly predictive of lower suicidality.

To our knowledge, this is the first study to examine the relationship between suicidality and resilience longitudinally. Our result of an inverse relationship between global resilience and suicidality is in agreement with and extends the results of the few cross-sectional studies that have previously examined this relationship (Green et al., 2010; Lockwood & Youssef; Nruham et al., 2010; Pietrzak et al., 2010; Roy et al., 2011). One of these cross-sectional studies is by Roy et al., who examined the relationship between suicidality and resilience. Their sample included 20 abstinent substance abuse patients who had attempted suicide, and another sample of 166 prisoners who had attempted suicide and matched control groups (Roy et al., 2011). In these samples, those who had never attempted suicide had significantly higher CD-RISC resilience scores compared with those who had attempted suicide. The authors reported that the study results “suggest that resilience may be a protective factor mitigating the risk of suicidal behavior associated with childhood trauma” (Roy et al., 2011).

Another study included 272 Iraq and Afghanistan veterans who were assessed cross-sectionally with a survey that contained measures of resilience. Thirty-four participants (12.5%) reported that they were contemplating suicide in the 2 weeks prior to the survey. Suicide contemplators had lower scores of resilience on CD-RISC. The investigators also found that suicide risk associated with childhood trauma was associated with lower resilience. Of relevance to our study, the investigators found that “sense of purpose and control were negatively associated with suicidal ideation” (Pietrzak et al., 2010).

Moreover, a study by Green et al. showed that resilience is inversely related to PTSD in veterans and to functional response to PTSD. In another study by our group, resilience has been shown to mitigate the effect of depression and suicidal ideation in Iraq and Afghanistan war veterans cross-sectionally (Lockwood & Youssef).

Although a study by Nruham et al. (Nruham et al., 2010) examined this relationship in a longitudinal study, resilience was measured cross-sectionally. The study examined a subset sample of 252 participants from eighth and ninth grade. The participants were followed up after 1 year and reassessed 5 years later. Resilience was assessed using the CD-RISC only at the last visit of the study (T3). The investigators found high resilience to be negatively associated with suicide attempts even if the adolescents were victims of violent life events and had been depressed at age 15.

It should be noted that secure relationships were significantly correlated with lower suicidality in the bivariate and multivariate model. However, in the multivariate model, secure relationships contributed to a small portion of the variance of approximately 13%. It is not clear why that is so and needs to be further validated in future studies. Possible explanations could be moderation due to other variables. Potential interacting and moderating effects that are worth examining in future studies are sex, age, and clinical variables such as PTSD diagnosis (Carter et al., 2011; Youssef et al., 2013).

Several investigators have stated that longitudinal studies are needed to increase the robustness of the relationship between resilience and suicidality, and to examine the relationship across time (Sarchiapone, Carli, Cuomo, & Roy, 2007). The current study fills this gap and confirms the previous finding, suggesting the protective effect of resilience across time. This is the first study to examine the relationship between suicidality and global resilience longitudinally, and the first to examine the factors of resilience individually across time. Interestingly, our longitudinal study results suggest that low resilience was a stronger predictor of suicidality than alcohol misuse and PTSD.

Increasing the confidence that this relationship between resilience and suicidality still holds across time paves the way for development of further programs and interventions to address and boost resilience as an important target for reducing suicidality above and beyond reducing risk factors for suicidality.

These interventions would best address psychosocial aspects that can bolster resilience, improve acceptance to change, and improve relationships. In addition, initial clinical trials of psychopharmacological agents have provided early evidence that resilience can be enhanced pharmacologically (J. Davidson et al., 2008; J. R. Davidson et al., 2005). In addition, an exploratory study has found pretreatment resilience to be a predictor of positive treatment response to the antidepressant venlafaxine (J. R. Davidson et al., 2012). Therefore, further examination of this line of research might lead to important interventional modalities for both preventive and treatment purposes above and beyond symptom control.

However, these results should be interpreted with caution in light of the study's limitations. For example, the possibility of other confounding effects could not be eliminated because of the observational design of the study. Potential confounders such as schizophrenia or bipolar disorder diagnoses also are limitations of this observational study. However, these disorders generally have a low base rate among military veterans because they are screened out during military admission evaluations. Another limitation of the present study is that it included a convenience sample of those who completed the interviews and rating scales. Baseline assessment consisted of self-selected veterans. As a result, participants never were initially approached at baseline. With respect to the follow-up, about one-quarter of initial patients had inaccurate contact information and could not be followed. Another limitation is that suicidality (suicidal ideation and attempts)—not completed suicide—was examined in this study. However, assessment of suicidality is the standard of clinical care and a proxy for assessing the risk of suicide (American Psychiatric Association, 2003). Also, a potential limitation is confounding due to psychotropic medications because of the FDA warning of suicidality for antidepressants and anticonvulsants. However, the contribution of antidepressants and anticonvulsants to suicidality (beyond the suicidality due to the primary mental illness) recently has been called into question from multiple rigorous studies (Gibbons, Brown, Hur, Davis, & Mann, 2012; Gibbons, Hur, Brown, & Mann, 2009, 2010; Isacsson, Holmgren, Osby, & Ahlner, 2009; Isacsson, Reutfors, Papadopoulos, Osby, & Ahlner, 2010; Isacsson, Rich, Jureidini, & Raven, 2010; Oquendo et al., 2011).

Despite these limitations, initial results from this longitudinal observational study are clinically informative and encouraging and would inform further studies that replicated this work. These results suggest that comprehensive assessment of resilience and its factors among veterans can contribute to understanding their clinical status in terms of suicidality and can inform clinical care. These may be important steps toward development of targeted preventive therapeutics programs, especially toward suicide prevention. In addition, the results could help direct future programs and therapeutics to bolster resilience and reduce suicidal ideation and attempts. Both the Department of Veterans Affairs and the Department of Defense have efforts under way to potentially increase resilience among active duty soldiers (Reivich, Seligman, & McBride, 2011) and veterans (Bates et al., 2010).

CONCLUSION

This is the first longitudinal study to establish the protective effect of resilience in veterans with suicidality. However, further longitudinal studies may increase confidence in these findings if the results are replicated in a larger sample and in other populations. Also, further research in this area with candidate interventions is still needed.

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Table 1**Demographics and clinical characteristics at baseline**

Variable	Total sample (N=176)
	Mean (SD)
Age, years	39 (10.6)
Education post high school (%)	57%
Sex, male (%)	82%
Race, white (%)	63%
Days between baseline and follow-up visit	1057 (174)
Suicidality	
Baseline BSS	0.50 (1.9)
Follow-up BSS	0.55 (2.3)
Resilience (CD-RISC)	76.7 (15.7)
Alcohol use disorder (AUDIT)	8%
PTSD (SCID)	16%

AUDIT: Alcohol Use Disorders Identification Test; BSS: Beck Scale for Suicide Ideation; CD-RISC, Connor Davidson Resilience Scale; PTSD: posttraumatic stress disorder; SCID: Structured Clinical Interview for DSM IV-Research Version; SD: standard deviation.

Table 2**Spearman correlation coefficients between suicidality and resilience factors**

Variables	r_s	P
Tenacity (factor 1)	−0.15	.06
Tolerance (factor 2)	−0.16	.049
Secure relationships (factor 3)	−0.34	<.0001
Control (factor 4)	−0.29	.0004
Spiritual (factor 5)	−0.10	.22

Table 3**Forward stepwise regression of the total sample for resilience factors on follow-up suicidality^a**

Variable	Partial R^2	Model R^2	C(p)	F value	P value
Baseline BSS	0.09	0.90	19.93	16.73	<.0001
Baseline secure relationships ^b	0.04	0.13	13.92	7.52	.007

a All variables left in the model are significant at the 0.05 level. No other variable met the 0.05 significance level for entry into the model.

b Secure relationships factor on the Connor Davidson Resilience Scale (factor 3).

BSS: Beck Scale for Suicide Ideation.

Table 4

Forward stepwise regression of the total sample for baseline resilience score on follow-up suicidality (BSS)^a

Variable	Partial R^2	Model R^2	C(p)	F value	P value
Baseline BSS	0.09	0.09	19.65	16.43	<.0001
Baseline CD-RISC	0.01	0.17	8.61	4.02	.047

^aAll variables left in the model are significant at the .05 level. No other variable met the .05 significance level for entry into the model.

The other variables included in the model were education, sex, ethnicity, race, PTSD, alcohol misuse on the AUDIT, and major depressive disorder. These variables (except sex) did not meet the .05 significance level for entry into the final model. Thus, resilience was a stronger predictor than PTSD or alcohol for suicidality.

AUDIT: Alcohol Use Disorders Identification Test; BSS: Beck Scale for Suicide Ideation; CD-RISC: Connor Davidson Resilience Scale; PTSD: posttraumatic stress disorder.

Nagy Youssef's contributions (added for the purpose of this thesis): Nagy Youssef was one of the fellow investigators at the Mid-Atlantic MIRECC when he was at Duke and Durham VA. He interviewed research patients, supervised SCID and PCL administration, collected the data with other MIRECC investigators, supervised research assistants and reviewed and edited this paper. He also worked with the statistician to guide the statistical analysis of this paper along with the other coauthors. He wrote the first draft of this manuscript and edited subsequent drafts. All authors contributed to the editing and conceptualization of this paper.

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Chapter 6 Racial/Ethnic Differences in the Association of Childhood Adversities With Depression and the Role of Resilience

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Abstract

Background: Adverse childhood experiences (ACE) including childhood abuse and trauma increase depressive symptoms. The role of resilience and how it interacts with both ACEs and the potential development of depressive symptoms, including how race and ethnicity moderate these effects, are much less studied. The aims of this study were to examine: 1) whether there is a dose-response relationship between trauma and depressive symptoms; 2) whether early trauma affected European Americans (EA) and African Americans (AA) in a similar fashion; and 3) whether resilience mitigates the effect of trauma.

Methods: The present study comprised a cross-sectional study of subjects from a longitudinal cohort. All subjects were 19 years or older with traumatic experiences prior to age 18. Subjects were assessed for depressive symptoms as well as resilience.

Results: In 413 subjects enrolled, ACEs were significantly associated with depression severity in a dose response fashion ($p < 0.001$). Notably, AAs had lower depression scores at low to moderate levels of ACEs than EAs, but reported comparable levels of depression with severe exposure to ACEs ($p_{\text{Interaction}}=0.05$). In both EAs and AAs, young adults with high and medium levels of resilience showed less depressive symptoms compared to those with low resilience ($p < 0.05$).

Limitations: The cross-sectional design, possibility of other confounders, and potential for recall bias of this study.

Conclusion: While ACEs were significantly associated with severity of depression in a dose-response fashion, higher resilience mitigated the impact of childhood adversities on depressive symptoms in young adults. The results are encouraging, and guides research for therapeutics to boost resilience.

Keywords

Adverse childhood experiences, Depressive symptoms, Resilience, Race/ethnicity

INTRODUCTION

Adverse childhood experiences (ACEs), characterized by childhood maltreatment and household dysfunction, have been associated with multiple physical health impairments in adulthood, including heart disease, obesity and type 2 diabetes (Felitti et al., 1998). Trauma exposure during early life has also been related to mental health issues later in life, including depression (Schulz et al., 2014; Wingo et al., 2010; Youssef, Green, Dedert, et al., 2013) and suicidality (Youssef, Green, Beckham, & Elbogen, 2013). For example, Youssef et al. found that childhood trauma exposure was significantly associated with both depressive symptoms and suicidal ideation in 1488 military personnel and veterans while controlling for the effects of combat exposure and posttraumatic stress disorder (PTSD) (Youssef, Green, Dedert, et al., 2013). Resilience, defined as qualities that enable one to thrive in the face of adversity, (Connor & Davidson, 2003) has been negatively associated with depressive symptoms and suicidal ideation, suggesting a protective effect (Youssef, Green, Beckham, et al., 2013; Youssef, Green, Dedert, et al., 2013). However, the role of resilience and how it interacts with both ACEs and the potential development of depressive symptoms, including how race and ethnicity moderate these effects, are much less studied.

The aims of the present study were to examine the following: 1) whether there is a dose-response relationship between number of trauma exposure in early life and depressive symptoms in young adulthood; 2) whether early trauma affected European Americans (EA) and African Americans (AA) in a similar fashion; and 3) whether resilience mitigates the effect of early trauma on the development of depression.

METHODS

Subjects

The present study is comprised of subjects from a longitudinal cohort that was established in 1989 to study the development of cardiovascular risk factors. All the subjects were recruited from the southeastern United States, who met the following criteria: (1) aged 5 to 16 years in 1989; (2) African or European ancestry; and (3) apparently healthy based on parental report of the child's medical history. Participants were classified as AAs if both parents reported being of African heritage and they considered themselves and their child to be AA, black, or Afro-American. Participants were classified as EAs if both parents reported that they were of European ancestry and they considered themselves and their child to be EA, white, or Caucasian, and not of Hispanic, Native American, or Asian descent. The study design and selection criteria have been described previously (Su et al., 2015).

At visit 15 (initiated at 2008), all subjects were 19 years or older and their traumatic experiences prior to age 18 were assessed by using the ACE questionnaire. Of 432 who were enrolled at visit 15, 413 completed the ACE questionnaire, including 192 EAs (99 males and 93 females) and 221 AAs (93 males and 128 females). There were no demographical differences (including age, gender, and ethnicity) between subjects who were included in the analysis (N=413) and those excluded (N=19) due to missing values.

Demographics Measurements

Demographic data were collected. Childhood socioeconomic status (SES) was assessed by Hollingshead Four Factor Social Status Index on the basis of parental education level and

occupation (Hollingshead, 1975). The Hollingshead scores ranged from 14 to 66, with a higher value indicating a higher SES.

Assessment of Adverse Childhood Experiences

The assessment of participants' exposure to ACEs covered the first 18 years of their lives. The ACE questionnaire consists of 28 items divided into 3 categories and 10 subscales, including childhood abuse (emotional, physical and sexual), neglect (emotional and physical), and growing up with household dysfunction (substance abuse, mental illness, domestic violence, criminal household member, and parental marital discord). The definition of ACEs has been described previously (Su et al., 2015). The ACE score (the number of 10 ACE subscales reported) was used to assess the cumulative effect of multiple ACEs, by classifying respondents into four groups: no exposure (0 ACEs, N=126), mild (1-2 ACEs, N=157), moderate (3-4 ACEs, N=75) and severe (≥ 5 ACEs, N=55) exposure.

Assessment of Depressive Symptoms

The Beck Depression Inventory (BDI) was used to assess depressive symptoms, a standardized scale providing a continuous measure of depressive symptoms (Beck, Steer, & Brown, 1996). This self-report instrument includes 21 items. Participants rate the severity of each symptom from 0 to 3. It has been used extensively in community samples and has satisfactory test-retest and internal consistency reliability.

Assessment of Resilience

Resilience was assessed using the Connor Davidson Resilience Scale (CD-RISC), which is a 25-item self-reported instrument that measures the ability to cope with stress and adversity, such as self-confidence, self-efficacy, self-control, optimism and spirituality/autonomy (Connor & Davidson, 2003). The items are scored on a five-point Likert scale, ranging from "not true at all" (scored 0) to "true nearly all the time" (scored 4). The scale rates participants over the past month with a total score of the CD-RISC varying from 0 to 100, with higher scores reflecting higher resilience.

Statistical Analysis

All analyses were done using STATA software. Sociodemographic variables were characterized with descriptive statistics (t-test for continuous traits and χ^2 -test for categorical traits). Multiple linear regression models were used to estimate the associations of ACE scores and resilience (CD-RISC scores) with depressive symptoms (BDI scores). Covariates included age, race, sex, BMI and childhood SES (Hollingshead index). The ethnicity and gender differences were further tested by including the interaction of ACE scores with race and sex, respectively. Statistical significance required a two-sided p-value of ≤ 0.05 . We conducted *a priori* power calculation using the G*Power software (Faul, Erdfelder, Buchner, & Lang, 2009). At a two-tailed α level of 0.05, the required sample size is N=408 to achieve a sufficient power of 0.8 for identifying an effect size as small as 0.027 (Cohen f^2). Therefore, the current study (N=413) should have sufficient power to identify small to medium effects.

RESULTS

A total of 413 subjects were included in the present study. The mean age (\pm SD) was 28.3 ± 3.1 (age range: 19.4 – 36.8). Table 1 presents the descriptive characteristics of the subjects by ethnicity and gender. African Americans (AA) had significantly greater BMI and lower childhood SES than European Americans (EA) ($p\leq0.001$). Men had lower BMI than women ($p=0.003$). The mean score on the BDI (\pm SD) was 6.7 ± 7.7 , with a minimum of 0 and a maximum of 39. The mean score on the CD-RISC (\pm SD) was 75.5 ± 13.9 , with a minimum of 27 and a maximum of 100. No significant differences on the BDI score or CD-RISC score were found between AAs and EAs, or between men and women.

Prevalence of ACEs

The prevalence of each individual ACE subscale and ACE scores are shown in Table 2, with the lowest prevalence of physical neglect (9.0%) and the highest prevalence of parental marital discord (38.7%). Compared to women, men reported much higher rate of experiencing physical neglect during childhood (15.1% versus 3.6%). Significant racial differences are evident in some of the ACE subscales (Table 2). Approximately 1 in 3 EA respondents reported that they lived with a household member who was mentally ill or attempted suicide, a rate twice as high as the rate reported by AA respondents (35.4% versus 17.2%). However, AA respondents reported higher rates of other ACE subscales than EA respondents. For example, 1 in 8 AA respondents reported experiencing physical neglect as a child, a rate 2 times higher than the rate reported by EA respondents (12.2% versus 5.2%). AA respondents also reported higher rates of (a) witnessing domestic violence (24% versus 17.2%), (b) a household member who went to prison (21.3% versus 9.9%) and (c) parental marital discord (47.1% versus 29.2%). Overall, the prevalence of any ACE event is relatively higher in AAs than that in EAs (73.3% versus 65.1%, $p=0.07$). In summary, 69.5% of respondents reported at least one exposure to ACEs, with mild (1-2 ACEs), moderate (3-4 ACEs), and severe (≥ 5 ACEs) exposure to ACEs representing 38.0%, 18.2% and 13.3%, respectively (Table 2). The prevalence of ACE scores was not significantly different between men and women, although men showed a higher rate of severe exposure to ACEs than women (16.6% versus 10.4%). AAs had a relatively higher prevalence of moderate and severe exposure to ACEs compared to EAs (21.3% versus 14.6% and 14.9% versus 11.4% respectively).

Effects of ACEs on depressive symptoms

The effects of each individual ACE on depressive symptoms are shown in Table 3 by gender and race. This table presents beta coefficients from regression models in which BDI score was regressed on one ACE, controlling for age, gender, race, BMI and childhood SES. Seven of ten ACEs were significantly associated with reports of higher depressive symptoms in both men and women, as well as in EAs and AAs. Women were more vulnerable to emotional neglect compared to men (beta coefficients: 8.0 versus 2.5; interaction $p=0.02$), while men reported higher depressive symptoms than women if some household member went to prison (beta coefficients: 5.2 versus 1.1; interaction $p=0.03$). The most frequently experienced ACE – parental marital discord – was not related to depressive symptoms in any gender or race group. The impacts of ACEs on depressive symptoms were generally stronger in EAs than in AAs for a given ACE, although they were not statistically different.

However, the cumulative effects of ACEs on depressive symptoms were significantly different between EA and AA respondents (interaction $p=0.05$). As shown in Table 4, after

controlling for age, gender, BMI and childhood SES, the EA participants who reported mild, moderate, and severe exposure to ACEs (1-2, 3-4, 5+) had an average increase of 2.1, 7.3 and 11.1 points on BDI scores compared to those with no exposure to ACEs ($p=0.05$, <0.001 and <0.001 respectively), while the AA participants at the same ACE exposure levels showed an average increase of 0.8, 3.3 and 12.6 points on BDI scores compared to their counterparts without ACEs ($p=0.5$, 0.02 and <0.001 respectively). There was no significant gender-by-ACE interaction, suggesting that the cumulative effect of ACEs on depressive symptoms was similar between men and women.

Role of resilience

We next examined the role of resilience in the relationship between ACE exposure and depression. For a visual conceptualization of the interaction between childhood adversities and resilience, we divided resilience into 3 categories based on the percentile of the CD-RISC score: high resilience: CD-RISC score >75 th percentile, medium resilience: CDRISC score >25 th percentile and ≤ 75 th percentile, and low resilience: CD-RISC score ≤ 25 th percentile. We found a significant interaction between ACE scores and resilience on depressive symptoms ($p<0.05$). Given similar levels of childhood adversity exposure, in both EAs and AAs, the high and medium resilience group had lower BDI scores compared to the low resilience group (Figure 1), suggesting that resilience mitigates the tendency for developing depressive symptoms in young adults with a history of childhood adversities. There is no three-way interaction of race, ACE scores and CD-RISC scores on depressive symptoms, indicating that resilience is a protective factor in both EA and AA populations.

DISCUSSION

This study has several findings that were expected and some that were not. We found that exposure to ACEs was significantly associated with severity of depression in a dose-response fashion. Given similar levels of childhood trauma exposure, young adults with high and medium levels of resilience showed less depressive symptoms compared to those with low resilience, as previously reported in both active duty service members and veterans (Youssef, Green, Dedert, et al., 2013). Our results replicated and generalized previous findings to the civilian young adult population.

However, an interesting finding in this study is that depression was only markedly higher in AAs with severe ACEs but not in mild or moderate ACEs; showing a significant interaction by race ($p=0.05$). AAs in general have less depressive scores at low to moderate level of ACEs than EAs, except for the severe exposure to ACEs where both races were more comparable in the severity of depression.

Our results are consistent with previous literature in several ways. For example, in a community-based ethnically diverse cohort of young adults in South Florida, Turner and Lloyd observed that AAs had generally experienced more adverse events than other ethnic groups but showed relatively lower prevalence of depressive disorders (Turner & Lloyd, 2004). In a longitudinal survey in young adults, Schilling et al. found that when racial/ethnic differences exist, young EAs consistently exhibit greater vulnerability to ACEs (Schilling, Aseltine, & Gore, 2007). One possible explanation is the potential social or cultural differences in resilience between two ethnic groups (Rutter, 1996).

However, the overall resilience scores did not differ between the two ethnic groups. Further adjustment for resilience scores did not change the results, suggesting that this racial/ethnic difference may not be attributable to the social or cultural differences in resilience. Another possible explanation is that individuals from minority backgrounds may be less willing to disclose mental health symptoms, which are often stigmatized in AA communities (U.S. Department of Health and Human Services & U.S. Public Health Service, 2001). However, it is of note that AAs exhibited high depressive scores that were comparable to EAs as the number of stressful childhood events exceeds 5 or more, suggesting that high levels of cumulative stress can have fairly equivalent negative effect on mental health for both AAs and EAs.

The protective role of resilience against stress and depression has been reported previously. For instance, a cross-sectional study of 792 predominantly African American men and women found that resilience moderated depressive symptom severity in individuals exposed to childhood abuse or other trauma (Wingo et al., 2010). Consistent with these findings, we also observed a significant interaction between ACE scores and resilience on depressive symptoms. In both EAs and AAs, young adults exposed to severe ACEs but with high levels of resilience showed less depressive symptoms compared to those with low resilience, suggesting that resilience mitigates the tendency for developing depressive symptoms in young adults with a history of childhood adversities.

There are several limitations to consider when interpreting the results of this study. First, given the cross-sectional design, the results cannot be interpreted in a cause-effect fashion, but only associations can be shown. Second, due to the observational design, the possibility of other potential confounding factors cannot be totally eliminated. Third, the potential for recall bias of past childhood experiences is inevitable in this type of study. However, good test-retest reliability of recall of past childhood trauma, and stability over time has been shown (Dube, Williamson, Thompson, Felitti, & Anda, 2004). Examination against documented cases of childhood trauma showed that inconsistencies are likely to be due to under-reporting rather than over-reporting of the trauma (Della Femina, Yeager, & Lewis, 1990; Dube et al., 2004; Hardt & Rutter, 2004). However, we did not collect information of an exact time frame when the childhood traumatic events occurred.

CONCLUSION

Exposure to ACEs was significantly associated with depression severity, while resilience mitigated the impact of childhood adversities on developing depressive symptoms in young adults. AAs exposed to low to moderate levels of ACEs had fewer depressive symptoms than EAs while at high levels of stress they exhibited similar severity of depression compared to EAs. This study provides useful information in the clinical assessment of patients with ACEs and depression. The results of our study are encouraging, and guide further research for promising therapies to boost resilience and potentially decrease depressive symptoms.

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Table 1

Descriptive characteristics of study participants (total N=413).

Characteristic	Men	Women	P_{gender}	EA	AA	P_{race}
N	192	221		192	221	
Age, y	28.2 (3.3)	28.5 (2.9)	0.45	28.0 (3.0)	28.7 (3.1)	0.025
Body mass index, kg/m ²	29.2 (6.6)	31.5 (9.8)	0.005	29.0 (7.8)	31.7 (9.0)	0.002
Hollingshead index	41.9 (13.9)	40.6 (13.1)	0.33	45.2 (12.3)	37.7 (13.6)	< 0.001
Depression symptoms (BDI score)	6.6 (7.8)	6.8 (7.7)	0.77	6.5 (7.4)	6.9 (8.0)	0.60
Resilience (CD- RISC score)	75.4 (14.6)	75.7 (13.3)	0.86	74.4 (13.4)	76.5 (14.3)	0.12

Means and standard deviations (SD) are shown.

EA: European American; AA: African American; BDI: Beck Depression Inventory; CD-RISC: Connor-Davidson Resilience Scale.

Table 2
Prevalence of adverse childhood experiences (ACEs) by race and gender.

ACE	All (N=413)	Men (N=99)	Women (N=93)	<i>P_{gender}</i>	EA (N=93)	AA (N=128)	<i>P_{race}</i>
	%	%	%		%	%	
Abuse							
Emotional	13.6	14.7	12.7	0.54	11.1	15.8	0.16
Physical	17.8	21.1	14.9	0.11	15.3	19.9	0.22
Sexual	16.1	16.8	15.5	0.72	15.2	16.8	0.65
Neglect							
Emotional	11.4	12.5	10.4	0.50	10.9	11.8	0.79
Physical	9.0	15.1	3.6	<0.001	5.21	12.2	0.013
Household Dysfunction							
Substance abuse	27.1	29.2	25.3	0.38	25.5	28.5	0.49
Mental illness	25.7	26.6	24.9	0.70	35.4	17.2	<0.001
Domestic violence	20.8	21.4	20.4	0.80	17.2	24.0	0.09
Criminal household member	16.0	16.2	15.8	0.93	9.9	21.3	0.002
Parental marital discord	38.7	42.2	35.8	0.18	29.2	47.1	<0.001
Any ACE	69.5	69.8	69.2	0.90	65.1	73.3	0.07
ACE score							
0	30.5	30.2	30.8	0.90	34.9	26.7	0.07
1-2	38.0	36.5	39.4	0.54	39.1	37.1	0.68
3-4	18.2	16.7	19.4	0.46	14.6	21.3	0.08
5+	13.3	16.6	10.4	0.06	11.4	14.9	0.30

EA: European American; AA: African American.

Table 3
Individual effects of adverse childhood experiences (ACEs) on depressive symptoms by race and gender.

ACE	Men			Women			EA		AA		
	Beta (SE)	P_{men}		Beta (SE)	P_{women}	$P_{Interaction}$	Beta (SE)	P_{EA}	Beta (SE)	P_{AA}	$P_{Interaction}$
Abuse											
	6.7(1.6)	< 0.001		8.3(1.4)	< 0.001	0.37	9.8(1.5)	< 0.001	6.3(1.4)	< 0.001	0.09
	5.6(1.4)	< 0.001		6.1(1.4)	< 0.001	0.73	7.6(1.4)	< 0.001	4.7(1.3)	< 0.001	0.13
Sexual	4.8(1.5)	0.001		7.8(1.3)	< 0.001	0.15	7.8(1.3)	0.001	5.3(1.4)	0.002	0.15
Neglect											
	2.5(1.7)	0.15		8.0(1.6)	< 0.001	0.02	5.5(1.7)	< 0.001	5.2(1.7)	< 0.001	0.87
Emotional	9.4(1.5)	< 0.001		9.3(2.7)	0.001	0.93	9.2(2.3)	< 0.001	9.1(1.6)	< 0.001	0.77
Physical											
Household Dysfunction											
	5.0(1.2)	< 0.001		4.7(1.1)	< 0.001	0.86	5.7(1.1)	< 0.001	4.0(1.2)	0.001	0.24
	4.7(1.2)	< 0.001		3.9(1.2)	0.001	0.75	4.3(1.0)	< 0.001	4.6(1.4)	0.001	0.90
	6.5(1.4)	< 0.001		4.7(1.2)	< 0.001	0.42	3.4(1.4)	0.01	7.0(1.2)	< 0.001	0.15
	5.2(1.5)	0.001		1.1(1.4)	0.45	0.03	5.2(1.7)	0.003	2.3(1.3)	0.09	0.10
Parental marital discord	1.0(1.2)	0.39		1.1(1.1)	0.33	0.88	0.01(1.2)	0.99	1.6(1.1)	0.14	0.41

SE: standard error; EA: European American; AA: African American.

Each model includes only one ACE subscale as predictor and controls for age, gender, race, BMI and childhood SES.

Table 4
Cumulative effects of adverse childhood experiences (ACEs) on depressive symptoms by race and gender.

ACE Score	Men			Women			EA			AA		
	Beta (SE)	P_{men}		Beta (SE)	P_{women}		Beta (SE)	P_{EA}		Beta (SE)	P_{AA}	
0	Reference			Reference			Reference			Reference		
1-2	1.2(1.2)	0.316		1.7(1.1)	0.114	0.59	2.1(1.0)	0.05		0.8(1.2)	0.50	0.05
3-4	5.4(1.5)	0.001		4.7(1.3)	< 0.001		7.3(1.4)	< 0.001		3.3(1.4)	0.02	
≥5	11.7(1.6)	< 0.001		13.4(1.6)	< 0.001		11.1(1.6)	< 0.001		12.6(1.6)	< 0.001	

SE: standard error; EA: European American; AA: African American

Each model includes ACE score as a categorical variable and controls for age, gender, race, BMI and childhood SFS.

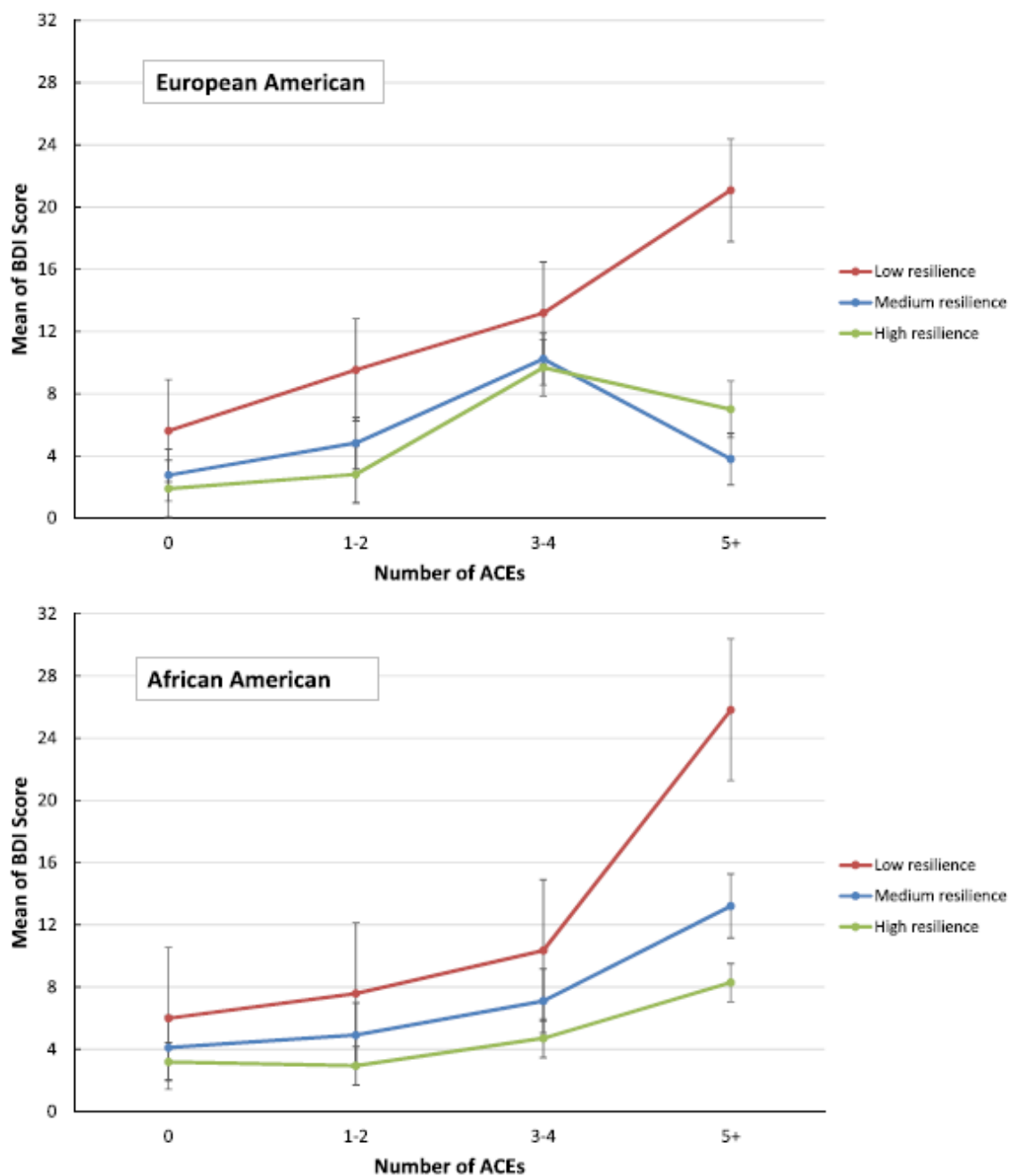


Fig. 1. Depressive symptoms according to ACE scores by Conner-Davidson resilience scale (total score ≤ 25 th percentile, > 25 th and < 75 th percentile, and ≥ 75 th percentile) and race (European American or African American).

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Chapter 7 Moral Injury, Post-Traumatic Stress Disorder, and Religious Involvement in U.S. Veterans

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Abstract

Background: Traumatic experiences can cause ethical conflicts. “Moral injury” (MI) has been used to describe this emotional/cognitive state, and may either contribute to the development of PTSD or block its recovery. We examine the relationship between MI and PTSD, and the impact of religious involvement (RI) on that relationship.

Methods: Study of 120 veterans enrolled at the Charlie Norwood VA Medical Center in Augusta, Georgia. Standard measures of PTSD symptoms, MI, and RI were administered. Regression models were utilized to examine correlates of PTSD symptoms and the moderating or mediating effects of RI.

Results: A strong relationship was found between MI and PTSD symptoms ($r=0.54$, $p<0.0001$), and between MI and RI ($r=-0.41$, $p<0.0001$), but only a weak relationship was found between RI and PTSD symptoms ($r=-0.17$, $p=0.058$). RI did not mediate or moderate the relationship between MI and PTSD symptoms in the overall sample. However, among non-Middle Eastern war theatre veterans, a significant buffering effect of religiosity was found.

Conclusions: MI has a strong positive relationship with PTSD symptoms and inverse relationship with RI. RI did not mediate or moderate the relationship between MI and PTSD in the overall sample, but moderated this relationship in non-Middle Eastern theatre veterans.

Keywords

Moral injury, post-traumatic stress disorder, spirituality, religiosity, veterans

INTRODUCTION

Moral injury (MI) has been described in military personnel following traumatic experiences during wartime (Litz et al., 2009; Nash & Litz, 2013; Shay, 1994). Litz and colleagues (2009) define it as “perpetrating, failing to prevent, bearing witness to, or learning about acts that transgress deeply held moral beliefs.” MI, a construct that is distinct from PTSD, has often been identified in those with PTSD, and may contribute to the onset and maintenance of PTSD (Dokoupil; H. G. Koenig et al., 2017). While PTSD is believed to be a chronic emotional and physical response to fear and danger, with hyper-alertness and physiological arousal, which can be triggered by reminders of the trauma, MI does not necessarily involve fear-based stressors (Nieuwsma et al., 2015). Instead, MI results from experiences that violate deeply held moral beliefs and values, which then arouses shame, guilt, anger, and existential questioning. Furthermore, MI symptoms include not only psychological symptoms (guilt, anger, shame, etc.), but spiritual symptoms as well (loss of meaning and hope, spiritual struggles, and loss of faith) (Harold G Koenig et al., 2017). Treatments targeting MI, both spiritual and secular, have been shown to significantly reduce PTSD symptoms (Gray et al., 2012; Ogden et al., 2011).

Because of MI’s connection with moral beliefs and values, religious beliefs/practices may either worsen symptoms of this condition (e.g., due to high moral standards that are more easily violated) or may help to relieve MI (e.g., through beliefs regarding forgiveness, rituals involving forgiveness/penance, and support through the faith community) (Worthington & Langberg, 2012). Religious beliefs and behaviors are important to many veterans, and are often used to cope with the stresses of war (H. G. Koenig et al., 1992; Harold G Koenig, Pargament, & Nielsen, 1998). Regarding the relationship of religious belief/involvement and PTSD, it may be postulated that being more religious may have a protective effect on PTSD, on the other hand some suggested that such association may be that having mental health problems causes people to be less religious.

However, religious involvement (RI) has been shown in veterans to predict faster recovery from depression (H. G. Koenig et al., 1992), greater post-traumatic growth (Tsai, El-Gabalawy, Sledge, Southwick, & Pietrzak, 2015), and faster recovery from PTSD (Currier, Holland, & Drescher, 2015). On the other hand, religious struggles and loss of faith as a result of combat or combat-related experiences have been associated with lack of full recovery from PTSD and continued need for VA mental health services (Currier, Holland, & Drescher, 2015; Fontana & Rosenheck, 2004; Witvliet, Phipps, Feldman, & Beckham, 2004). Ogden and colleagues (Ogden et al., 2011) also found that PTSD symptoms were associated with alienation from God, religious rifts, religious fear, and religious guilt among veterans of Middle Eastern wars. For the purpose of this study, Middle Eastern wars included OEF/OIF/OND veterans, that is to say the Middle Eastern region (Operation Iraqi Freedom, Operation New Dawn, and other Middle Eastern conflicts), as well as Afghanistan (Operation Enduring Freedom). Non-Middle Eastern wars included Vietnam, Korea, WWII era veterans, and miscellaneous other combat theatres.

MI may be particularly problematic for a certain subgroup of veterans, particularly younger veterans who served in Iraq/Afghanistan, and who may possibly experience more

religious struggles (raised within a culture with high moral values, but perhaps fewer religious resources to cope with those moral struggles). Currier et al. (2014) compared community-dwelling adults of similar age and veterans who served in Iraq/Afghanistan and found less religiosity, more religious struggles, and weaker spirituality in the latter group. Thus, RI may either predispose veterans to the development of MI (and consequently worsen PTSD symptoms), may provide veterans with resources to help cope with and resolve MI (and consequently improve PTSD symptoms), or may buffer the effects of MI in generating and maintaining PTSD symptoms (blocking the negative effects of MI on PTSD). Further research is needed to better understand the potentially complex relationships and interactions between RI, MI, and PTSD symptoms.

Objectives

This study's objectives were to (1) examine the relationship between moral injury (MI) and severity of PTSD symptoms in U.S. military veterans, and (2) determine the mediating and moderating effects of religiosity on the relationship between MI and PTSD severity.

METHODS

This study was approved by the institutional review board (full review) and the Research and Development Committee of the Charlie Norwood VA Medical Center and by the Duke University Medical Center institutional review board (exempted). After informed consent, 120 veterans at the Charlie Norwood Veterans Administration Medical Center were recruited via advertisements and clinicians' referrals (from several mental health clinics including general mental health clinics and PTSD clinics) and completed a questionnaire that asked about demographics, military service information, physical health, use of alcohol, PTSD diagnosis, and information about religious affiliation, importance of religion and spirituality, and level of religious commitment. Inclusion criteria were being a veteran, deployed to a combat theater, and the presence of PTSD symptoms and/or symptoms of inner conflict. All participants gave written informed consent, and were compensated \$25 for their time in the form of a gift card.

Questionnaire

Demographics. Demographic information collected included age, gender, race (white, black, Hispanic, Asian, multiracial, other), education (years), and marital status (single, divorced, married, widowed).

Military Information. Participants were asked if they were involved in combat or served in a combat zone; the particular combat theatre that they were deployed to (Middle East, Vietnam, Korea/WWII, other); and the time since deployment (years).

PTSD Symptoms/Diagnosis. Participants were also asked if they had PTSD symptoms without a formal diagnosis or had a formal PTSD diagnosis. PTSD severity was assessed with the 20-item PTSD Checklist of the Department of Veterans Affairs, DSM-5 version (PCL-5) (Weathers et al., 2013). The PCL-5 assesses the symptoms of PTSD as required for a diagnosis of PTSD in DSM-5. Participants were asked, “How much have you been bothered by each problem in the past month.” Possible response options are 0 (“not at all”), 1 (“a little bit”), 2 (“moderately”), 3 (“quite a bit”), and 4 (“extremely”). Responses are summed to create a PTSD severity scale that ranges from 0 to 80. The 20-item PCL-5 has high reliability and strong associations with combat exposure, and functional impairment in military personnel (Ashbaugh, Houle-Johnson, Herbert, El-Hage, & Brunet, 2016; Hoge, Riviere, Wilk, Herrell, & Weathers, 2014).

Physical Health/Alcohol Use. Difficulty engaging in physical activity was assessed on a 0 to 10 scale (0=no difficulty, 10=great difficulty); similarly, average daily physical pain was assessed on a 0 to 10 scale (0=no pain, 10=severe pain). Finally, current alcohol use was inquired about, with possible responses ranging from “none” to “some” (2 drinks or less/day) to “quite a bit” (more than 2 drinks but less than 7 drinks per day) to “a lot” (>6 drinks/day).

Religious Involvement. Religious affiliation was assessed as Christian, Non-Christian (of other religious persuasion), or none/atheist/agnostic. Importance of religion and importance of spirituality in life were each measured on a 4-point Likert scale from 1 (not important at all) to 4 (very important). Religious commitment and overall involvement were assessed with the 10-item Belief Into Action (BIAC) Scale (Harold G Koenig, Nelson, et al., 2015; Harold G Koenig, Wang, Al Zaben, & Adi, 2015). The BIAC scale assesses degree of religious commitment, time spent in religious activity, and money given for religious causes, with responses ranging from 1 to 10 for each item. The internal reliability ($\alpha = 0.89$, 95% CI=0.86-0.91) and test-retest reliability for the BIAC after one week (ICC= 0.92, 95% CI=0.87-0.95) are acceptable, and the scale has solid convergent, discriminant, and factor analytic validity (one factor explaining 94% of variance).

Moral Injury (MI). The MI Symptoms Scale is a 54-item multidimensional measure that assesses symptoms of moral injury, and is composed of 10 subscales: guilt, shame, violation of moral values, self-condemnation, betrayal by others, loss of trust, difficulty forgiving, loss of meaning, purpose and hope, spiritual struggles, and loss of faith (Harold G Koenig et al., 2017). We have recently shown in a multicenter study in veterans and active duty military the reliability and validity of this multi-dimensional symptom measure of moral injury that can be used in studies targeting MI in veterans and active duty military personnel with PTSD symptoms, and may also be used by clinicians to identify those at risk (H. G. Koenig et al., 2017). The guilt subscale consists of two items taken from the Moral Injury Questionnaire (MIQ) (Currier, Holland, Drescher, & Foy, 2015) and three items from the Combat Guilt Scale (CGS) (Henning & Frueh, 1997) (standardized Cronbach’s alpha in the present sample=0.41). The shame subscale consists of two items from the CGS and an item from the work of Andrews with Veterans from the United Kingdom (Andrews, Brewin, Stewart, Philpott, & Hejdenberg, 2009) (alpha=0.65). The 3-item violation of moral values subscale consists of statements of moral transgression from the Moral

Injury Events Scale (MIES) (Nash & Litz, 2013) that assess commission and omission ($\alpha=0.79$). The self-condemnation subscale is made up of all 10 items of the Rosenberg Self-Esteem Scale (SES) (Rosenberg, 1965), which measures global self-worth by assessing both positive and negative feelings about the self ($\alpha=0.84$). The 3-item betrayal subscale from the MIES assesses feelings regarding betrayal by others during wartime ($\alpha=0.62$). Six items make up the loss of trust subscale, and these come from the General Trust Scale (GTS) that assesses beliefs about the honesty and trustworthiness of others (Yamagishi & Yamagishi, 1994) ($\alpha=0.80$). Difficulty forgiving is measured by a 10-item subscale consisting of six statements from the Heartland Forgiveness Scale (HFS) (Thompson et al., 2005) and four questions that ask about feeling forgiven by God, forgiving God, and forgiving self, and the need to seek forgiveness ($\alpha=0.69$). Loss of meaning, purpose and hope is assessed by a 7-item subscale, with five items coming from the Presence of Meaning subscale of the Meaning in Life Questionnaire (MLQ) (Steger, Frazier, Oishi, & Kaler, 2006), a sixth item coming from the MIQ, and a seventh item that asks how hopeful the individual is about the future ($\alpha=0.82$). Finally, religious/spiritual struggle is assessed by the 7-item subscale of the Brief RCOPE (Pargament, Smith, Koenig, & Perez, 1998) and loss of faith subscale by a single question that asks how faith has changed as a result of wartime experiences ($\alpha=0.87$).

All 54 items of the MI Symptom Scale are rated on a 1-10 scale in terms of agreement or disagreement to each statement (with reverse scoring of items so that higher scores indicate more symptoms of MI) producing a response ranging from 54 to 540. The average MI score in the present sample was 262.0 with a SD of 68.0 and range from 119 to 452. Overall, the MI Symptom Scale had an alpha of 0.92.

Missing Values. In constructing scales, missing items on the scales were handled as follows. If participants answered at least 50% of items on a scale or subscale, the average of items answered was substituted for the missing item score (done for the PCL-5 in 12% of cases, the BIAC in 3%, and the MI subscales in 0% to 13%). A similar procedure was followed for combining subscale scores to produce the overall MI Symptom Scale score. If an entire subscale score was missing, the average of completed subscale scores was inserted for the missing score (done in 7% of cases).

Statistical Analyses

Descriptive statistics were used to report characteristics of the sample with means (and standard deviations) and frequency distributions. Bivariate analyses were conducted using Pearson's r for continuous variables, Student's t -test for continuous variables across dichotomized categories, analysis of variance (ANOVA) for continuous variables across three or more categories, and chi-square for two categorical variables. Variables significant at $p<0.15$ in bivariate analyses were entered into general linear models predicting PTSD symptom severity that consisted of the MI Symptom Scale score alone (Model 1), followed by adding demographic

and military variables (Model 2), then physical health and behavioral variables (Model 3), then religious commitment (BIAC) (Model 4), and then the interaction between MI symptoms and BIAC score (to test the moderating effect of religiosity on the relationship between MI and PTSD symptom severity). For Models 4 and 5, the BIAC was dichotomized with two-thirds of the sample in the low religiosity category and one-third in the high category. Alpha level was set at $p=0.05$, and was not corrected for multiple comparisons due to the exploratory nature of these analyses. All statistical tests were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC).

RESULTS

Sample characteristics are displayed in Table 1. The average age of participants was 55.6 years with a range from 23 to 81 years. The majority of participants were male (86.4%), African-American (62.9%) or white Caucasian (30.2%), married (43.7%) or divorced (29.4%), and Christian (84.9%). Average education was 13.8 years with a range of 4 to 25 years. This sample contained veterans who were deployed. The majority were involved in combat (67.5%), served in Iraq, Afghanistan, or other Middle Eastern theatres (48.7%) or Vietnam (31.3%), and the average time since last deployment was 23.2 years (range 6 months to 60 years). The vast majority indicated they had a formal diagnosis of PTSD (93.1%), and the average PCL-5 score was 53.7 (where the cut-off for PTSD ranges from 28 to 38) (Ashbaugh et al., 2016). Most participants experienced some level of physical disability (average 6.1 on a 0 to 10 scale) and daily chronic pain (average 6.5 on a 0 to 10 scale). Religion and spirituality were “very important” to many (54.2% and 59.3%, respectively), and the average BIAC score was 47.1 (SD 20.9), similar to the average score found in stressed family caregivers of disabled persons in North Carolina (47.2, SD=21.5) (Harold G Koenig, Wang, et al., 2015).

Bivariate Correlations

As hypothesized, MI symptoms were strongly and positively correlated with PTSD symptom severity ($r=0.54$, $p<0.0001$) (Table 2). In contrast, MI symptoms were strongly and inversely correlated with religious involvement assessed by the BIAC ($r=-0.41$, $p<0.0001$). However, religiosity was only weakly correlated with PTSD symptom severity ($r=-0.17$, $p=0.058$). Other significant correlates of PTSD symptoms were higher education level, deployment to Afghanistan/Iraq or other Middle Eastern war theatres, and especially impairments in physical functioning ($r=0.40$, $p<0.0001$) or daily chronic physical pain ($r=0.27$, $p<0.005$). Compared to veterans serving in other combat theatres, MI and PTSD severity were both higher in veterans serving in the Middle East ($F=6.9$, $p=0.002$ and $F=2.4$, $p<0.10$, respectively), whereas religiosity (BIAC) was significantly lower ($F=5.36$, $p=0.006$).

Multivariate Analyses

The strongest independent predictor of PTSD symptom severity was MI symptoms (Table 3). Higher education and being married (surprisingly) were other significant independent predictors of PTSD symptom severity. Poorer physical functioning was also a significant predictor. Once MI symptoms were controlled for, religiosity score on the BIAC (either continuous or dichotomized) was no longer related to PTSD symptoms and did not mediate the relationship between MI symptoms and PTSD symptoms in the overall sample. Furthermore, the interaction between MI and BIAC scores was not significant, indicating no buffering effect for religiosity on the relationship between MI and PTSD symptom severity.

Given that both MI and PTSD symptoms were higher and religiosity lower in Middle East theatre veterans, further exploratory analyses were conducted. The buffering effect of religiosity on the relationship between MI and PTSD symptoms was examined separately in Middle Eastern theatre veterans and non-Middle Eastern theatre veterans (Vietnam, Korea, WWII, and other theatres). Given the small samples in these sub-analyses, only MI, BIAC score (continuous), and the interaction between MI and BIAC scores were examined as predictors of PTSD severity in these models. Among non-Middle Eastern theatre veterans (N=63, including 3 missing data for combat theatre), a significant interaction was found between MI and BIAC scores ($B=-0.004$, $SE=0.002$, $t=-2.09$, $p=0.04$), whereas this was not found in Middle Eastern theatre veterans (N=56) ($B=0.001$, $SE=0.001$, $t=0.80$, $p=0.43$). To determine what the significant interaction meant in non-Middle Eastern theatre veterans, we again dichotomized veterans in terms of high and low religiosity. Among highly religious non-Middle Eastern theatre veterans (N=31), no significant correlation was found between MI and PTSD symptom severity ($B=0.04$, $SE=0.05$, $t=0.68$, $p=0.50$), whereas among less religious non-Middle Eastern theatre veterans (N=32), a significant positive correlation between MI and PTSD symptoms was present ($B=0.14$, $SE=0.04$, $t=3.19$, $p=0.003$).

DISCUSSION

To our knowledge, this is the first study to explore the role of religious involvement in the established relationship between symptoms of moral injury resulting from wartime experiences and PTSD symptom severity. In the overall sample, we found that MI was strongly and positively correlated with severity of PTSD symptoms, although level of religious involvement or commitment had little or no effect on this relationship in the overall sample, neither mediating nor buffering it. However, in an exploratory analysis among non-Middle Eastern theatre veterans, who were typically older and more religious, high religiosity completely buffered the effects of MI on PTSD symptoms. However, there was no evidence for such a buffering effect among younger veterans who were deployed in recent operations in Iraq, Afghanistan, and other Middle East conflicts.

Other researchers have likewise reported a strong correlation between exposure to morally injurious events and PTSD symptoms or another psychopathology (A. O. Bryan, Bryan, Morrow, Etienne, & Ray-Sannerud, 2014; C. J. Bryan et al., 2016; Currier, Holland, Drescher, et al., 2015; Nash et al., 2013; Nickerson et al., 2015; Wisco et al., 2017). However, moral injury scales used to examine these correlations assessed a mixture of traumatic experiences (not amenable to treatment) and emotional symptoms (treatable), and they did not assess spiritual symptoms of moral injury (e.g., religious struggles or loss of faith). While no studies (to our knowledge) have examined the buffering effect of religiosity on the relationship between MI and PTSD symptoms, several studies with larger samples of veterans have similarly found a significant inverse correlation between religious involvement and PTSD symptoms (Currier, Drescher, & Harris, 2014; Currier, Holland, & Drescher, 2015; Hourani et al., 2012; Sharma et al., 2017). Likewise, religiosity has also been shown to buffer the effects of traumatic experiences on mental health outcomes in veterans and non-veterans (Bentley, Ahmad, & Thoburn, 2014; Chang, Skinner, & Boehmer, 2001; Chang, Skinner, Zhou, & Kazis, 2003; Korn & Zukerman, 2011).

While the present study found a weak inverse relationship between religiosity and PTSD symptom severity in bivariate analyses, this relationship lost its borderline significance when other factors were controlled for in the multivariate analyses. Although religiosity was inversely related to MI, it did not buffer the effects of MI on PTSD symptoms in the overall sample. However, religiosity buffered the effects of MI on PTSD symptoms among non-Middle Eastern theatre veterans. Thus, it appeared that religiosity among veterans of Vietnam, WWII, Korea, and other non-Middle Eastern theatres helped to block the effects of MI on PTSD symptoms or to promote recovery when such difficulties emerged, whereas religiosity had little effect on this relationship among veterans serving in Iraq, Afghanistan, or other Middle Eastern theatres. The latter group also tended to be less religious, yet have higher MI and PTSD symptom scores. Although difficult to explain, perhaps religiosity was less effective in helping Middle Eastern theatre veterans cope with the moral injuries that they had suffered, thus inducing or increasing the severity of their PTSD symptoms.

These findings, if verified in future studies, have clinical implications. MI involves not only psychological symptoms such as guilt and shame, but also spiritual symptoms such as religious struggles and loss of religious faith. Neither symptom type can be ignored in any comprehensive treatment for those with PTSD. Psychotherapies, both spiritually-integrated and secular forms, are needed to target the psychological and the spiritual symptoms of moral injury or inner ethical conflict. Based on the present findings, spiritual therapies for MI in the setting of PTSD may be particularly effective in older veterans who have served in non-Middle Eastern war theatres. The limitations of the present study, however, make such conclusions preliminary.

Limitations

Several study characteristics limit the generalizability and interpretability of these findings. First, the cross-sectional nature prevents any speculations on direction of causation in the relationships identified. Second, this was a relatively small sample and one that was drawn from a single state (Georgia), one located in the Bible Belt (although many of these veterans were not originally from Georgia). Third, there may be some overlap between our measure of religious involvement (BIAC) and the spiritual dimensions of moral injury (MI), which needs to be acknowledged; however, these dimensions are quite distinct since spiritual struggles may occur in those who are religious or in those who are not religious, and often there is little relationship between the two (as has been found for positive and negative religious coping). Finally, significant buffering effects of religiosity were identified only in exploratory analyses, and significance levels were not corrected for multiple comparisons. However, the strengths of this study are the use of psychometrically valid comprehensive measures of MI symptoms (symptoms that are amenable to change), PTSD symptom severity, and religious commitment, along with careful statistical control for demographic, military, and physical health confounders.

CONCLUSIONS

While symptoms of moral injury appeared to be strongly and positively correlated with severity of PTSD symptoms (the strongest of any correlates identified here), level of religious involvement in the overall sample did not influence this relationship--neither mediating nor buffering it. However, among veterans serving in non-Middle Eastern combat zones, religiosity appeared to buffer the effects of moral injury on PTSD symptom severity. Future studies are needed that longitudinally follow active-duty military from prior to deployment to discharge from the service, prospectively examining the effects of baseline religiosity on MI and PTSD symptoms, taking into account the severity of wartime trauma and confounding factors such as pre-service mental health and physical injuries experienced during combat. Randomized clinical trials are also needed to determine whether interventions that specifically target MI in those with PTSD result in a faster remission of PTSD symptoms and its comorbidities compared to standard therapies used today.

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TABLE 1
Characteristics of the sample (N = 120)

	% (n) / Mean (SD) (range)
Age, years	55.6 (14.8) (23 to 81)
Sex (% female)	13.6% (16)
Race (% black)	62.9% (73)
Education, years	13.8 (3.2) (4 to 25)
Marital status (% married)	43.7% (52)
Involved in combat (% yes)	67.5% (79)
Combat theater	
Middle Eastern	48.7% (56)
Vietnam	31.0% (36)
Other	20.7% (23)
Time since deployment, years	24.2 (17.3) (0.5 to 60)
Diagnosis of PTSD (% yes)	93.1 (108)
Physical functioning, average on 0 to 10 scale	6.1 (2.6) (0 to 10)
Pain severity, average on 0 to 10 scale	6.5 (2.4) (0 to 10)
Alcohol use (% >2 drinks/day)	13.8% (16)
Religious affiliation	
Christian	84.9% (101)
Non-Christian	2.5% (2)
None, agnostic, atheist	12.6% (15)
Importance of religion (% very)	54.2% (65)
Importance of spirituality (% very)	59.3% (70)
Belief into Action Scale score	47.1 (20.9) (10 to 93)
PCL-5 score	53.7 (15.3) (15 to 80)
MI symptom score	262 (68.0) (119 to 452)

N may vary by up to 5%.

MI: moral injury; PCL-5: PTSD Symptom Checklist DSM-5 version;

PTSD: posttraumatic stress disorder; SD: standard deviation.

TABLE 2
Sample characteristics and PTSD symptoms
(PCL-5) (bivariate correlations)

Demographic	<i>r</i>	PCL-5 (mean, SD)	<i>P</i>
Age, years	-.11		.23
Education, years	.26		<.01
Race			
Black		54.0 (14.2)	.96
Other		54.2 (16.9)	
Marital status			
Married		55.9 (13.2)	.14
Other		51.7 (16.8)	
Military			
Involved in combat			
Yes		54.7 (15.2)	.35
No		51.8 (16.0)	
Combat theater			
Middle Eastern		56.6 (14.8)	.04
Other		50.9 (15.4)	
Years since deployed	-.10		.30
Diagnosis of PTSD			
Yes		54.7 (14.9)	.02
No		40.7 (19.6)	
Physical health/health behaviors			
Physical functioning (0 to 10)	.40		<.0001
Pain severity (0 to 10)	.27		<.005
Alcohol use			
>2 drinks/day		58.8 (16.8)	.15
<3 drinks/day		52.8 (15.2)	
Religious			
Christian			
Yes		52.9 (15.2)	.19
No		58.1 (15.6)	
Religious importance			
Very important		53.1 (14.9)	.66
More than very		54.4 (15.8)	
Spiritual importance			
Very important		52.4 (14.8)	.31
More than very		55.4 (16.2)	
BIAC score (religiosity)	-.17		.058
MI Symptom score	.54		<.0001

BIAC: Belief Into Action Scale; MI: moral injury; PCL-5: PTSD Checklist DSM-5 version;
SD: standard deviation.

TABLE 3

Predictors of PTSD severity (PCL-5) (multivariate)

	Model 1 beta (SE)	Model 2 beta (SE)	Model 3 beta (SE)	Model 4 beta (SE)	Model 5 beta (SE)
MI	.12 (.02) ^a	.13 (.02) ^a	.12 (.02) ^a	.12 (.02) ^a	.12 (.02) ^a
Demographic and military					
Education, years	...	1.17 (.36) ^b	1.13 (.36) ^c	1.14 (.36) ^c	1.13 (.36) ^c
Marital status (married)	...	6.56 (2.33) ^c	5.62 (2.40) ^d	5.69 (2.41) ^d	5.69 (2.43) ^d
Combat theater (Middle Eastern)	...	-.37 (2.42)	-.95 (2.49)	-1.68 (2.68)	-1.69 (2.69)
Physical health/behaviors					
Physical functioning (0 to 10)	1.07 (.54) ^d	1.11 (.55) ^d	1.10 (.55) ^d
Pain severity (0 to 10)28 (.57)	.34 (.58)	.34 (.58)
Alcohol use (>2 drinks/day)87 (3.49)	.70 (3.51)	.77 (3.55)
Religious					
BIAC score (dichotomized)	-2.10 (2.81)	-.30 (10.27)
Interaction (MI × BIAC)	-.01 (.04)
Model R-square (n)	.29 (120) ^a	.42 (110) ^a	.46 (107) ^a	.47 (107) ^a	.47 (107) ^a

Variables included in multivariate model only if $P < .15$.

BIAC: Belief into Action Scale; MI: moral injury; PCL-5: PTSD Checklist DSM-5 version; PTSD: posttraumatic stress disorder; SE: standard error.

^a $P \leq .0001$.

^b $P \leq .001$.

^c $P \leq .01$.

^d $P \leq .05$.

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Chapter 8 Relapse Prevention After Index Electroconvulsive Therapy in Treatment-Resistant Depression

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Abstract

Background: One-third of patients who suffer from depression are resistant to conventional treatments. An acute course of electroconvulsive therapy (ECT) can lead to remission of depressive symptoms in a substantial portion of the treatment-resistant patients. However, prevention of relapse with depressive symptoms after an index course of ECT can be challenging. We reviewed pertinent studies on the topic and analyzed the best strategies to avoid relapse and recurrence of depressive symptoms.

Methods: We performed a systematic literature review of PubMed through April 2014 for clinical trials published in English to determine if continuation ECT, continuation medication, continuation psychotherapy, or combinations of these are the best strategy to avoid relapse and recurrence of depressive symptoms after an acute course of ECT. Clinical trials comparing ≥ 2 of the above strategies were included in the review.

Results: Although there are few rigorous randomized clinical trials in this area, most studies suggest that combined continuation ECT (C-ECT) and continuation pharmacotherapy are the most effective strategy in relapse prevention.

Conclusions: C-ECT and continuation pharmacotherapy may be more effective than either alone for preventing relapse. However, more definitive randomized clinical trials are needed.

INTRODUCTION

Approximately 20.9 million American adults a year suffer from a mood disorder (Kessler, Chiu, Demler, Merikangas, & Walters, 2005). Major depressive disorder (MDD) is the most prevalent mood disorder, and is the leading cause of disability among Americans age 15 to 44 (World Health Organization, 2008). It also is a risk factor for mortality including death due to suicide and other medical conditions. One-third of patients do not respond to pharmacotherapy (including medication combinations) and psychotherapy (Rush et al., 2006). Among treatment-resistant patients, >100,000 patients per year in the United States are treated with electroconvulsive therapy (ECT)(Dukakis & Tye, 2007); relapse after ECT is common (Coppin et al., 1981), however, and strategies for reducing the risk of relapse after ECT are under-studied. This review will focus on prevention of relapse or recurrence of depressive episodes of mood disorders after a successful acute course of ECT (depressive episodes being the most common indication for ECT).

Psychotherapeutic interventions such as cognitive-behavioral therapy can help patients with mild to moderate depressive disorders and should be tried early in treatment. Psychopharmacological agents are indicated for severe depression (Davidson, 2010). Unfortunately, many patients do not respond or experience remission while taking psychopharmacological agents. For example, the response rate to a first antidepressant is 50%, and remission is only 37%.

Definition of response, remission, and relapse in ECT trials

In antidepressant trials, treatment response often is defined as a reduction in Hamilton Rating Scale for Depression (HRSD) Score of $\geq 50\%$ (Frank et al., 1991). In psychotherapy trials, treatment response often is defined similarly to pharmacotherapy trials (Driessen et al., 2013). However, in ECT clinical trials, response often is defined as reduction in HRSD-24 of $\geq 60\%$ after an ECT course. The definition of remission in many ECT trials is symptom reduction of $\geq 60\%$ on HRSD scores and a final HRSD score ≤ 10 (Petrides et al., 2001; Sackeim et al., 2001; Sackeim et al., 2000).

Some studies (Prudic, Olfson, Marcus, Fuller, & Sackeim, 2004) had 2 definitions of remission: “moderate and strict criteria.” In Prudic et al., the “moderate criteria (remitter10)” is a reduction of $\geq 60\%$ on HRSD scores and a final HRSD score of ≤ 10 . The “strict criteria (remitter7)” is a reduction of $\geq 60\%$ on HRSD scores and a final HRSD score of ≤ 7 (Prudic et al., 2004).

Relapse in clinical trials often is defined as the return of full syndromal symptomatic criteria. Alternatively, relapse is defined as a HRSD score of ≥ 16 and an absolute increase of 10 points for at least 1 week (over 2 consecutive visits) (Sackeim et al., 2001).

As mentioned, the response rate to the first antidepressant is approximately 50% and the remission rate is 37% (Rush et al., 2006). This rate falls with each successive antidepressant trial,

reaching 14% after the third trial and 13% after the fourth trial according to the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial (Rush et al., 2006). A total of 33% of patients do not achieve symptomatic remission (let alone functional recovery) despite multiple trials of medication (including augmentations with multiple medications)(Rush et al., 2006). These represent millions of Americans each year who do not experience remission of depression, (Kessler et al., 2003; Kessler et al., 1994) and are deemed treatment-resistant. Of the patients that are resistant to conventional therapies, approximately 60% responded to ECT (Franco-Bronson, 1996; McCall, 2001).

After a successful acute course of ECT, maintaining remission from depressive symptoms is a major challenge for clinicians and patients because the relapse rate has been reported to be as high as 84% within the first 6 months after ECT (Sackeim et al., 2001). To address this challenge, we performed a systematic review of the literature to best inform the course of continuation treatment.

METHODS

A systematic literature review of PubMed was performed through April 2014 for clinical trials published in English to determine whether continuation ECT, continuation medication, continuation psychotherapy, or combinations of these are the best strategy to avoid relapse and recurrence of depressive symptoms after an acute course of ECT. Clinical trials comparing ≥ 2 of the above strategies were included in this systematic review. Keywords used were (ECT OR electroconvulsive therapy OR convulsive therapy) AND (depression OR depressive disorder OR depressive episode) AND (maintenance OR continuation).

RESULTS

The options for continuation treatment are: (1) pharmacotherapy, (2) psychotherapy, (3) continuation ECT, or (4) a combination of the above interventions.

Continuation pharmacotherapy with or without continuation psychotherapy

A common practice after an index course ECT is to prescribe antidepressants for continuation therapy (Abou-Saleh & Coppen, 1988). Although earlier controlled trials of continuation medication alone (C-MED) reported encouraging results, (Kay, Fahy, & Garside, 1970; Seager & Bird, 1962) C-MED alone is not effective for all patients, with some studies showing high relapse rates (Prudic et al., 2004; Rasmussen et al., 2007; Sackeim et al., 1990).

Combinations of psychotropics might be better for preventing relapse than a single drug. When a combination of ≥ 2 drugs is used, lithium augmentation seems to lead to better results than antidepressants alone. This has been shown in a well-designed randomized, double-blinded, placebo-controlled clinical trial that enrolled 290 participants. Of these participants, 159 met

remission criteria, and 84 (who remitted and agreed to participate) were randomized to 3 arms after remission after acute ECT (Sackeim et al., 2001). Participants were randomized to the continuation arms for 24 weeks after depressive remission to receive nortriptyline alone, a combination of nortriptyline and lithium, or placebo. The study found that a combination of nortriptyline and lithium reduced the relapse rate (39%) compared with nortriptyline monotherapy (60%) or with placebo (84%) over 6 months (Sackeim et al., 2001). Of note in this study, every relapse using nortriptyline-lithium occurred before the 5-week period except for 1 patient.

In another controlled trial after successful index ECT, C-MED resulted in a relapse rate of 51% within 6 months for treatment as usual, 60% for protocolized care with nortriptyline, and 39% for protocolized care with nortriptyline plus lithium (39%), versus a relapse rate of 84% for placebo (Tew et al., 2007). This study included 56 randomized patients and was single-blinded.

In clinical practice, personalized treatment decisions are based on clinical, demographic, and logistic characteristics, with consideration of predictors of response, predictors of relapse, patient preferences, and long-term adverse effects. Of note, although cognitive adverse effects can be cause for concern in some cases during an acute course of ECT (especially bilateral ECT), cognitive side effects during C-ECT are not as much of a concern because the sessions are not timed as closely together.

Logistic concerns include the availability of a family or friend who can transport the patient to continuation ECT (C-ECT) sessions and the ability to take time-off (if working) for C-ECT. Factors that predict higher likelihood of relapse after an acute course of ECT are in Table 1. Relapse prevention strategies are in Table 2.

Continuation ECT (C-ECT)

Because the difference between maintenance ECT and C-ECT is arbitrary, for the purpose of this article we will refer to both as C-ECT. C-ECT indicates any treatment session after the index course administered with an intention to prevent relapse or recurrence of depressive symptoms rather than to induce response/remission of an episode.

Relapse rates with placebo continuation therapy after index ECT are high (Sackeim et al., 2001). Several studies verify the benefits of C-ECT in sustaining remission (Sackeim et al., 1990; Spiker, Stein, & Rich, 1985). This might be helpful, especially in cases where patients did not respond to several pharmacological agents (Sackeim et al., 1990), are sensitive to medication side effects, or prefer ECT over medication.

A controlled, well-designed trial in this area led by Kellner and colleagues (Consortium for Research in ECT, CORE) evaluated the comparative efficacy of C-ECT vs combination C-MED (lithium carbonate plus nortriptyline) in preventing depressive relapse over a 6-month period after successful bilateral (BL) ECT for acute depression. This randomized, controlled clinical trial enrolled 201 participants in the phase II comparison of C-MED vs C-ECT. The study was single-

blinded for neuropsychological tests. This study found equal sustained remission rate of 46% throughout the study period for both the C-MED and the C-ECT arm of the study (Kellner et al., 2006).

In a recent meta-analysis and systematic review of literature on post-ECT relapse that included 32 studies, the authors reported that in modern studies of C-MED, 51% of patients relapsed by 12 months after a successful acute course of ECT. A total of 37.7% of patients who relapsed did so within the first 6 months. The relapse rate at 6 months with C-ECT was similar to C-MED (37.2%) (Jelovac, Kolshus, & McLoughlin, 2013).

Another recent literature review on C-ECT concluded that ECT is “an underused treatment option that can substantially reduce risks of relapse”(Rabheru, 2012). The factors favoring consideration of C-ECT (with or without pharmacotherapy) are listed in Table 3.

Combination of C-ECT and pharmacotherapy or psychotherapy

A general principle with antidepressant therapy is that treatment for the acute phase also is effective as continuation treatment. However, after acute response to ECT, the most common clinical practice is to stop ECT and to use only medications as continuation therapy (Aronson, Shukla, & Hoff, 1987) despite the fact that some patients who undergo ECT have not responded to medications or psychotherapy before ECT.

Long-term remission may be preserved with combined C-ECT and pharmacotherapy, especially when symptoms did not respond to medication before ECT. Retrospective studies of hospitalization rates have reported lower rates of psychiatric hospitalization in patients receiving C-ECT with medications compared with admission rates before receiving ECT alone (Russell et al., 2003; Thornton, Mulsant, Dealy, & Reynolds, 1990).

Another retrospective study comparing C-ECT plus medication (C-ECT group) versus C-MED alone (C-MED group) demonstrated a significant advantage for the C-ECT group (Gagne, Furman, Carpenter, & Price, 2000). The 2-year cumulative probability of surviving without relapse or recurrence for the ECT group was 93% while the 2-year cumulative probability of surviving without relapse or recurrence was only 52% for the C-MED group. At 5 years, the C-ECT group had relapse-/recurrence-free survival of 73%, while the C-MED group had relapse-/recurrence-free survival of 18%. The weaknesses of this study included its retrospective nature. Although on the high end for ECT studies, sample size was modest (N=59) to allow for adjustments for some of the potential confounding variables. Because of the retrospective nature, there was no random assignment of the groups or blinding. The mean survival time for staying well for the C-ECT group was 6.9 years, while for the C-MED group it was 2.7 years (Gagne et al., 2000). These results support the superior efficacy of C-ECT plus medications compared with C-MED alone in preventing relapse and recurrence in patients with depression who have responded to acute ECT.

A randomized trial by Brakemeier et al. (Brakemeier et al., 2013) examined the most efficacious continuation intervention for patients who responded to acute ECT. After response—not remission—to acute right unilateral (RUL) ultra-brief ECT, 60 patients were randomized to cognitive-behavioral therapy (C-CBT) plus “guideline-based” C-MED, C-ECT (RUL ultra-brief) plus C-MED, or C-MED alone. The study found superiority in the C-CBT group on the proportion of patients who “remained well” after 12 months (primary outcome). In this study, 70% of the initial 90 patients responded to acute ECT, and 47% remitted. After 6 months, sustained response rates were 77% in the C-CBT arm, 40% in the C-ECT arm, and 44% in the C-MED alone arm. After 12 months, sustained response rates were 65% in the C-CBT group, 28% in the C-ECT group, and 33% in the C-MED alone group. The authors concluded that “ultra-brief pulse ECT as a continuation treatment correlates with low sustained response rates,” and that C-CBT plus C-MED “might be an effective continuation treatment to sustain response after successful ECT in MDD patients”.

During the initial acute ECT phase, participants were switched to BL ECT if they did not respond to RUL ECT. Nonetheless, during the randomization continuation phase, all participants in the C-ECT arm were given RUL ECT. It is not clear how many of the participants who had BL ECT were treated later with RUL ECT during the continuation phase, but this could make ECT appear to be ineffective during the continuation phase, because the BL ECT that was previously effective was discontinued. For the above reasons, it is hard to draw conclusions on the superior efficacy of C-CBT compared with the ultra-brief unilateral ECT used in this study without more rigorous replication of this study.

Another small prospective study examined 21 participants who received C-ECT (12 depressed and 8 schizoaffective) and 21 matched controls (received C-MED) who refused C-ECT. This study compared patients who received C-ECT plus pharmacotherapy with those who received C-MED alone. Limitations of this study include small sample size, no random assignment, no blinding, and the C-ECT group was not homogenous including both RUL and BL ECT and mixed diagnoses. However, the study was prospective and had a matched control group. The study found a significantly better outcome for patients receiving C-ECT plus pharmacotherapy compared with C-MED alone in terms of time to rehospitalization (Swoboda, Conca, Konig, Waanders, & Hansen, 2001).

Another prospective, randomized, single-blind trial assessed tolerability and efficacy of continuation treatments for geriatric patients with psychotic depression after acute ECT remission (Navarro et al., 2008). The researchers compared the 2-year period outcome of 2 groups of geriatric patients with psychotic major depression who remitted after acute treatment of ECT plus nortriptyline. During the continuation phase, one group was randomized to nortriptyline alone (C-MED) (N=17), and the other group was randomized to C-ECT plus nortriptyline (N=16) (C-ECT). Over the 2 years of treatment, the mean survival time of remission was significantly longer for the combined C-ECT plus C-MED group compared with the C-MED group. There was no difference in tolerability between the 2 groups. The researchers concluded

that the study supported the use of combined C-ECT plus antidepressant treatment in geriatric patients with psychotic major depression who remitted using acute ECT.

Another prospective, multicenter, randomized, controlled trial tested the “hypothesis that relapse prevention with continuation ECT plus pharmacotherapy is more effective than pharmacotherapy alone” after a course of acute ECT for major depressive episode (unipolar or bipolar depression)(Nordenskjold et al., 2013). The study randomized 2 parallel groups (after response to acute ECT) in 4 hospitals in Sweden. Participants (N=56) were randomly assigned to either 29 C-ECT sessions with C-MED or C-MED alone for 1 year. The C-MED consisted of antidepressants (98%), lithium (56%), and antipsychotics (30%). The primary outcome was relapse within 1 year. (Relapse was defined as ≥ 20 points on the Montgomery-Åsberg Depression Rating Scale or inpatient psychiatric care or suicide or suspected suicide.) Sixty-one percent of participants relapsed within 1 year in the C-MED alone arm versus 32% of those in the C-ECT plus C-MED arm ($P = .036$). (The Cox proportional hazard ratio was 2.32 (95% confidence interval, 1.03–5.22).) In the C-MED arm, there was 1 suspected suicide by intoxication (died from intoxication, the suicidal intent was not proven but was considered in the study as a suspected suicide) and 3 suicide attempts but none in the combined C-ECT arm. The authors concluded that there was a “statistically significant advantage for combined treatment with pharmacotherapy and continuation ECT”.

The Prolonged Remission in Depressed Elderly (PRIDE) study is an ongoing multicenter, randomized clinical trial by the CORE group mentioned above. The PRIDE study examines whether combined pharmacotherapy (lithium plus venlafaxine) plus C-ECT, or combined lithium plus venlafaxine C-MED alone, is more effective in maintaining remission in depressed older patients with MDD after acute ECT. Data from this study will be valuable in informing clinical design-making of combining ECT with these medications.

In summary, most studies have shown that combined C-ECT plus continuation pharmacotherapy (C-MED) have significantly better results in relapse prevention than either alone (Navarro et al., 2008; Nordenskjold et al., 2013; Swoboda et al., 2001). One study showed that C-CBT combined with C-MED is better than C-ECT combined with C-MED. However, it is hard to draw conclusions or recommend C-CBT as superior to ECT based on a single study because of design limitation, and results that are different from most of the literature.

Other considerations in continuation ECT and pharmacotherapy in clinical practice

There are other important points that need to be considered in the routine clinical aspects of continuation/maintenance therapy. First, it is helpful to have a rating scale to consistently and systematically assess improvement or relapse in depressive symptoms during acute ECT and continuation treatment. Although many clinical trials have used HRSD, clinicians can use simpler, self-administered scales, including the 9-item Patient Health Questionnaire (Spitzer, Kroenke, & Williams, 1999).

Second, clinicians should individualize treatment decisions by weighing risks and benefits of psychotropics, especially lithium, among other alternatives for treatment-resistant depressive episodes of unipolar and bipolar disorders. Lithium can have several side effects including nausea, thyroid dysfunction, and renal dysfunction, and blood level monitoring (for lithium level, renal, and thyroid functions) is required. However, if used with caution and blood tests are checked regularly, most patients tolerate the drug well (Mauri et al., 1999). The risk from chronic lithium use includes renal toxicity (McCann, Daly, & Kelly, 2008). If kidney function is monitored closely, however, lithium generally can be used safely in most cases, even for long periods of time (McCann et al., 2008; Tredget, Kirov, & Kirov, 2010). Also, a systematic review and meta-analysis of 23 studies concluded that “any lithium-associated increase in serum creatinine is quantitatively small and of questionable clinical significance” (Paul, Minay, Cardwell, Fogarty, & Kelly, 2010). However, routine monitoring of renal function is essential. Lithium augmentation plays an important role for managing treatment-resistant depression (Bauer et al., 2010). It has potential benefits not only in treatment-resistant depression (Licht, 2012), but also in reducing suicide (Schou, 1999) in bipolar and unipolar depression (Cipriani, Hawton, Stockton, & Geddes, 2013; Muller-Oerlinghausen, Berghofer, & Ahrens, 2003). The drug also has a neuroprotective effect (Chiu & Chuang, 2011).

Third, although cognitive side effects—which can be of concern during acute ECT—are a possibility, they usually improve with time. Acute side effects of ECT have been discussed extensively elsewhere (American Psychiatric Association (2000)) and are not the focus of this article. For the purposes of this article, C-ECT has less cognitive side effects than acute ECT, simply because of the timing of treatment sessions. Among C-ECT patients, there likely is additional cognitive sparing as the treatment sessions are moved farther and farther apart. However, although this represents our clinical experience and the general consensus in the field, it has not been verified by empirical research. There are no studies comparing cognitive effects of ECT with session spacing during C-ECT, for example between patients getting ECT once per week versus once per month.

Fourth, one of the barriers to pharmacotherapy is the high rates of medication non-adherence (Lopez-Torres, Parraga, Del Campo, & Villena, 2013). Adherence to antidepressant treatment may range from 30% to 70% (Barbui et al., 2000). A study by Cramer and Rosenheck (Cramer & Rosenheck, 1998) examining pooled data reports that adherence rates for antidepressants rarely exceed 65%. Others found 37% of patients discontinued antidepressants by 12 weeks in the counselled group compared with 61% in the non-counselled group (Peveler, George, Kinmonth, Campbell, & Thompson, 1999). Another study found that only 20% to 34% of individuals filled ≥ 4 prescriptions (Katon, von Korff, Lin, Bush, & Ormel, 1992).

Physicians cannot predict medication adherence in many patients (Lopez-Torres et al., 2013). Non-adherence is commonly encountered in outpatient care not only with older medications such as tricyclic antidepressants, but also with newer medications such as selective serotonin reuptake inhibitors (Barbui et al., 2000). However, studies indicate that adherence for

C-MED might be lesser than outpatient C-ECT. Dew and McCall found an outpatient C-ECT adherence rate of 91% (Dew & McCall, 2004). This could be attributed to ECT being a more potent and rapidly acting form of treatment for treatment-resistant depressive episodes and/or suicidality.

As directions for further research, we recommend that further investigations in refinements of C-ECT and continuation pharmacotherapeutic combinations—and psychotherapy especially in patients who were non-treatment resistant to psychotherapy—with ECT are needed to further improve relapse prevention strategies after index ECT, especially for treatment-resistant mood disorders.

CONCLUSIONS

Although there are few rigorous randomized clinical trials in this area, the current literature suggests that combined C-ECT and pharmacotherapy may be more effective than either alone in preventing relapse. However, more definitive randomized clinical trials to guide the best strategies to avoid relapse after an acute course of index ECT are needed.

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Table 1

Common relapse predictors

<ol style="list-style-type: none">1. Medication resistance2. Female sex3. Residual depressive symptoms4. Comorbid untreated psychiatric disorders such as posttraumatic stress disorder, substance use disorder, or personality disorder5. History of unsuccessful treatment with ECT if the treatment parameter and electrode placement were optimized6. Highly recurrent major depressive disorder7. Depressive symptoms as part of a rapid cycling bipolar disorder8. Uncontrolled medical condition that is inducing or exacerbating the mood disorder such as untreated hypothyroidism or chronic severe pain disorder
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Table 2

Relapse prevention strategies

1. Starting continuation pharmacotherapy during the index course
2. Aggressively treating residual symptoms after an index course of ECT
3. Tailoring C-ECT session to the level of symptoms and clinical presentation without over or under treatment, rather than using a fixed schedule for continuation ECT
4. Combined ECT and pharmacotherapy including possibility of lithium augmentation
5. Gradually discontinuing (or minimizing if absolutely necessary) benzodiazepine use and other CNS depressants to both avoid the depressogenic effect of these substances as well as to avoid interference with seizure induction during ECT
6. Treating other comorbid psychiatric conditions (formerly Axis I disorders) that might be hindering treatment such as posttraumatic stress disorder, substance use comorbidity etc.
7. Treating comorbid personality disorder that might be hindering treatment such as treating borderline personality disorder with dialectical behavioral therapy
8. Treating comorbid medical condition that could be exacerbating (or inducing) mood disorder such as treating hypothyroidism

C-ECT: continuation electroconvulsive therapy; ECT: electroconvulsive therapy.

Table 3

Factors favoring consideration of continuation ECT

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| <ol style="list-style-type: none">1. Positive response to index ECT2. Patient preference3. Failure of pharmacotherapy and psychotherapy to achieve remission in the past4. Intolerance to effective pharmacological agents or side effects unacceptable to the patient5. Limited medical illness that could increase risk of further ECT or anesthesia6. Trials to discontinue ECT result in rapid reemergence of depressive symptoms despite optimization of pharmacological interventions and/or pharmacotherapy7. Potential for high stakes complications is there were a relapse, i.e., return of intense suicidality or catatonia8. No barriers to the logistic issues in complying with outpatient ECT (such as transportation and availability of a friend or family to be involved in care; ability to take a day off for the ECT procedure, if applicable) |
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ECT: electroconvulsive therapy.

Table 4

Prospective clinical trials and meta-analysis involving combining C-ECT with C-MED (or C-CBT)

Study	Design, N	Interventions	Treatment details/ECT/anesthesia	Primary outcome measure(s)	Baseline	Endpoint/outcomes	Comment
Nordenskjöld 2013 ³⁵	Multicenter, randomized (2 parallel groups), non-blinded, controlled trial	After response to acute ECT for MDE (unipolar or bipolar depression) participants were randomized (N = 56) to 29 C-ECT sessions with C-MED (n = 28) or C-MED alone (n = 28) for 1 year. The C-MED consisted of antidepressants (98%), lithium (56%), and antipsychotics (30%)	ECT Weekly ECT for 6 weeks, then every 2 weeks for 46 additional weeks (total of 29 ECT sessions). UL UB ECT (6 times ST) was used. Meda Spectrum 5000Q device at 3 hospitals and a Thymatron System IV (Somatics, Inc., Lake Bluff, Ill) was used at one hospital. Propofol or thiopental was used as anesthetic, and succinylcholine as muscle relaxant Pharmacotherapy Patients received individualized medication. Venlafaxine was first choice, with lithium augmentation offered to all patients. Antipsychotics were allowed. Pharmacotherapy at randomization consisted of antidepressants (98%), lithium (56%), and antipsychotics (30%). If the patient experienced a relapse, any treatment change was allowed, including ECT for all patients regardless of initial allocation	Relapse within 1 year. (Relapse was defined as ≥20 points on the MADRS, psychiatric hospitalization, suicide, or suspected suicide)	Responders (not remitters) to acute ECT randomized (N = 56)	61% relapsed within 1 year in the C-MED alone arm vs 32% of those in the C-ECT plus C-MED arm (P = .036). (The Cox proportional hazard ratio was 2.32 (1.03 to 5.22). In the C-MED arm there was 1 suspected suicide and 3 suicide attempts but not in the combined C-ECT arm	The authors concluded that there was a "statistically significant advantage for combined treatment with pharmacotherapy and continuation ECT"
Navarro et al 2008 ³⁴	Prospective, randomized, single-blind trial (N = 33) Patients age ≥60 with psychotic major depression (in a MDE) were recruited	After remission with acute ECT, one group was randomized to nortriptyline alone C-MED (n = 17), and the other group was randomized to C-ECT plus nortriptyline (n = 16) (C-ECT)	Acute ECT (N = 38) Bilateral ECT 3 times per week with MECTA-SR2 plus nortriptyline (to obtain blood level of 80 and 120 ng/mL [maximum dose: 100 mg/d]) until remission Anesthesia: Succinylcholine (40 to 100 mg), atropine (0.5 to 1 mg), and thiopental (200 to 300 mg) Continuation randomization phase (N = 33): C-ECT group (n = 16). C-ECT plus nortriptyline was given. After 1 week of the acute phase, weekly ECT session for 1 month was administered, then every 2 weeks for 1 month, and then monthly ECT stimulus and the anesthetics were similar to acute ECT. C-MED group (n = 17). Nortriptyline combined with risperidone (up to 2 mg/d) for 6 weeks, then withdrawn over a 4-week period	Time to relapse/recurrence at 2-years (secondary: HDRS-17)	Remitted (N = 33) after acute ECT randomized in phase II HDRS (considered remitters when the HDRS-17 score was < 8 at 3 consecutive ECT session)	Over 2 years of treatment in geriatric, psychotic, unipolar depressed ECT (plus nortriptyline) remitters, the mean survival time of remission was significantly longer for the combined C-ECT plus C-MED group (n = 12 completers: 11 Without relapse or recurrence) compared with the C-MED group (n = 13 completers: 5 Without relapse or recurrence). There was no difference in tolerability between the 2 groups	The researchers concluded the study supports combined C-ECT plus antidepressant treatment in geriatric patients with psychotic major depression who remitted with acute ECT

Study	Design, N	Interventions	Treatment details/ECT/anesthesia	Primary outcome measure(s)	Baseline	Endpoint/outcomes	Comment
Brakenmeier et al 2013 ²²	Single site, 3-arm parallel-group randomized study after acute ECT, 90 inpatients with MDD, including 60 who responded to acute ECT	Randomized (N = 60) C-MED+C-BT (n = 17) (n = 16 completers) C-MED+C-BT (n = 25) (n = 18 completers) C-MED alone (n = 18) (n = 14 completers)	C-ECT arm: Acute ECT: 3 times/week, with Mecta 5000Q device RUL UB ECT. If no improvement switched to BL ECT Anesthesia: propofol C-ECT: with RUL UB ECT C-CBT: Group CBT C-MED alone: In all groups, C-MED included a guideline medication algorithm. There was no standard medication for all patients	Proportion of patients who remained well at 12 months	Responders (not remitters) after acute ECT randomized (N = 60) in phase II	C-MED only group 14 completed 12-month response rates: 33% C-ECT group, 18 completed 12-month response rates: 28% C-CBT group 17 completed 12-month response rates: 65%	The authors concluded: "this study suggests, for the first time, that combining CBT with individualized antidepressant therapy is an intervention that increases sustained response and decreases dropout rates in depressive patients who have previously responded to ECT. The study provides, in addition, evidence that ECT with ultra-brief pulses in conjunction with propofol anesthesia as used here in a therapy-resistant population, significantly reduces continuation ECT efficacy. In sum, the study might be a useful initial step in a long-term psychotherapeutic treatment regime for patients after ECT"

BL: bilateral; ECT: electroconvulsive therapy; HDRS: Hamilton Depression Rating Scale; MADRS: Montgomery-Åsberg Depression

Rating Scale; MDE: major depressive episode; ST: seizure threshold; UB: ultrabrief pulse ECT; UL: unilateral.

Contributions (added for the purpose of this thesis): Nagy Youssef conceptualized the idea of this systematic review and developed the inclusion criteria and search strategy, searched the literature, and wrote the initial draft of the manuscript. Both Nagy Youssef and Vaughn McCall contributed intellectually and edited the later versions of the paper. Both coauthors approved the final version of the manuscript.

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Chapter 9 Effects of Continuation Electroconvulsive Therapy on Quality of Life in Elderly Depressed Patients: A Randomized Clinical Trial

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Abstract

We examined whether electroconvulsive therapy (ECT) plus medications (“STABLE + PHARM” group) had superior health-related quality of life (HRQOL) compared with medications alone (“PHARM” group) as continuation strategy after successful acute right unilateral ECT for major depressive disorder (MDD). We hypothesized that scores from the Medical Outcomes Study Short Form-36 (SF-36) would be higher during continuation treatment in the “STABLE + PHARM” group versus the “PHARM” group. The overall study design was called “Prolonging Remission in Depressed Elderly” (PRIDE). Remitters to the acute course of ECT were re-consented to enter a 6-month RCT of “STABLE + PHARM” versus “PHARM”. Measures of depressive symptoms and cognitive function were completed by blind raters; SF-36 measurements were patient self-report every 4 weeks.

Participants were 120 patients ≥ 60 years old. Patients with dementia, schizophrenia, bipolar disorder, or substance abuse were excluded. The “PHARM” group received venlafaxine and lithium. The “STABLE + PHARM” received the same medications, plus 4 weekly outpatient ECT sessions, with additional ECT session as needed. Participants were mostly female (61.7%) with a mean age of 70.5 ± 7.2 years. “STABLE + PHARM” patients received 4.5 ± 2.5 ECT sessions during Phase 2. “STABLE + PHARM” group had 7 point advantage (3.5-10.4, 95% CI) for Physical Component Score of SF-36 ($P < 0.0001$), and 8.2 point advantage (4.2-12.2, 95% CI) for Mental Component Score ($P < 0.0001$). Additional ECT resulted in overall net health benefit. Consideration should be given to administration of additional ECT to prevent relapse during the continuation phase of treatment of MDD.

Keywords

Electroconvulsive therapy; quality of life; randomized controlled trial; major depressive disorder; continuation therapy

TRIAL REGISTRATION: Clinical Trials.gov NCT01028508

INTRODUCTION

Major Depressive Disorder (MDD) is a leading cause of poor health-related quality of life (HRQOL)(World Health Organization, 2011). The HRQOL deficits increase with depression symptom severity (W. Vaughn McCall, Wayne Cohen, Beth Reboussin, & Powell Lawton, 1999). Age influences the HRQOL deficit patterns, with younger depressed patients reporting more problems with relationships and older depressed patients reporting more problems with daily living and role functioning (W. Vaughn McCall et al., 1999). A third of depressed patients do not respond to two or more sequential antidepressant medications and are deemed to have treatment-resistant depression (TRD) (Kubitz, Mehra, Potluri, Garg, & Cossrow, 2013; McCall, 2007). TRD patients are candidates for electroconvulsive therapy (ECT), acknowledged as the most effective TRD treatment (Lisanby, 2007).

HRQOL is exceptionally poor in MDD patients referred for ECT and worse than that of unselected MDD patients in general outpatient settings, (McCall et al., 2013) and HRQOL is a factor in referral patterns for ECT (W. V. McCall, W. Cohen, B. Reboussin, & P. Lawton, 1999). Naturalistic studies of MDD have shown that ECT results in improved HRQOL, with the degree of improvement greater for patients who received ECT as opposed to antidepressant medications (McCall, Reboussin, Cohen, & Lawton, 2001). Similarly, modern ECT randomized clinical trials (RCT) not including a non-ECT comparator arm also showed improvement in HQOL (McCall, Dunn, & Rosenquist, 2004; McCall et al., 2011). Both in the naturalistic studies and the prior RCTs, improvement in HRQOL was best explained by improvement in depression symptoms, with little or no relationship to any cognitive side effects. In naturalistic studies, improvement in HRQOL was sustained over 6-months after ECT in patients with sustained remission with HRQOL values indistinguishable from healthy population norms (McCall et al., 2013). In contrast, depressive relapse after ECT was associated with worsening in HRQOL (McCall, Prudic, Olfson, & Sackeim, 2006).

HRQOL is central to understanding the overall net risks and benefits of treatments, including those of ECT. While ECT results in remission of depressive symptoms, it is also associated with cognitive side effects. The issue of cognitive side effects is of particular concern for the elderly population who are more vulnerable to age-related cognitive problems (Rizzi, Rosset, & Roriz-Cruz, 2014). Medical decision-making regarding the risk/benefit ratio of ECT could be usefully informed by the study of health related quality of life measures (Devanand, Dwork, Hutchinson, Bolwig, & Sackeim, 1994; Scalia et al., 2007; Weiner, 1984). However, prior HRQOL studies in ECT have lacked randomized comparisons of ECT versus a non-ECT alternative group.

We present here the HRQOL outcomes as a secondary analysis from a randomized comparison of ECT combined with venlafaxine (VEN) and lithium (Li), versus VEN and Li without ECT, as continuation therapy after a successful ECT course for elderly adults with MDD.

MATERIAL AND METHODS

Design Overview

The *Prolonging Remission in Depressed Elderly (PRIDE)* study was a NIH-funded randomized, multi-center study that compared two post-acute-ECT continuation treatment strategies: (1) pharmacotherapy that combined venlafaxine (VEN) and lithium (Li) (*PHARM*); and (2) *PHARM* enhanced by the addition of an individualized, flexible, algorithm-based ECT schedule (**Symptom-Titrated, Algorithm-Based, Longitudinal ECT, STABLE**) (*STABLE + PHARM*) (Lisanby et al., 2008).

PRIDE consisted of two phases: in Phase 1, 240 patients, ≥ 60 years old with unipolar MDD received acute ECT 3 times per week in combination with oral VEN; in Phase 2, 120 remitters who were randomized to either *PHARM* or *STABLE + PHARM* comprised the intent-to-treat (ITT) sample. The primary efficacy outcome variable was the 24-item Hamilton Rating Scale for Depression (HRSD₂₄) total score measured longitudinally over 6-months. *A priori* secondary outcome variables included HRQOL. The results of Phase 1 have been previously reported for both antidepressant efficacy and HRQOL (Kellner, Husain, Knapp, McCall, Petrides, Rudorfer, Young, Sampson, McClintock, Mueller, Prudic, Greenberg, Weiner, Bailine, Rosenquist, Raza, Kaliora, Latoussakis, Tobias, Briggs, Liebman, Geduldig, Teklehaimanot, Lisanby, et al., 2016; McCall et al., 2017) and the Phase 2 efficacy results have been reported (Kellner, Husain, Knapp, McCall, Petrides, Rudorfer, Young, Sampson, McClintock, Mueller, Prudic, Greenberg, Weiner, Bailine, Rosenquist, Raza, Kaliora, Latoussakis, Tobias, Briggs, Liebman, Geduldig, Teklehaimanot, Dooley, et al., 2016). The study was approved by the institutional review board at each study site, and the investigation was carried out in accordance with the latest version of the Declaration of Helsinki.

Patient Sample

Patients enrolled in Phase 1 were aged 60 years and older and were referred for ECT for the treatment of unipolar MDD, without dementia, with or without psychosis, with a pretreatment HRSD₂₄ total score ≥ 21 . Exclusion criteria included: bipolar disorder, schizoaffective disorder, schizophrenia, dementia, delirium, intellectual disability, history of substance abuse in the past 6 months, or neurological conditions or active general conditions assumed to affect cognition or treatment response. Also, patients failing to respond to an adequate trial of Li+VEN or ECT in the current episode were excluded. Inclusion criteria for the randomized phase (Phase 2) were achievement of remission in Phase 1 defined as: (a) HRSD₂₄ total score ≤ 10 on two consecutive ratings, and (b) HRSD₂₄ total score did not increase > 3 points on the second consecutive HRSD₂₄ or remained ≤ 6 . Written informed consent was obtained before entrance to Phase 1 and before randomization in Phase 2.

Treatments

Symptom-Titrated, Algorithm-Based Longitudinal ECT (STABLE). *STABLE* featured an initial fixed, tapered, ECT treatment schedule followed by a symptom driven, flexible component, in addition to the same VEN + Li as in *PHARM*, and the combination is termed *STABLE + PHARM*. The initial fixed portion consisted of 4 ECT sessions in one month, within specified treatment windows. Treatment frequency in the subsequent flexible component (weeks 5-24) was

determined by application of the STABLE algorithm, which prescribed 0-2 ECT treatments in a given week based on a patient's HRSD₂₄ total scores, details of which have been previously reported (Lisanby et al., 2008).

ECT procedures. ECT was delivered with right unilateral electrode placement with a high-dose, ultrabrief pulse stimulus (RUL-UBP), described in our earlier report (Kellner, Husain, Knapp, McCall, Petrides, Rudorfer, Young, Sampson, McClintock, Mueller, Prudic, Greenberg, Weiner, Bailine, Rosenquist, Raza, Kaliora, Latoussakis, Tobias, Briggs, Liebman, Geduldig, Teklehaimanot, Dooley, et al., 2016). Continuation ECT in Phase 2 was administered at the same stimulus dose as the last treatment in Phase 1.

Medication Procedures. Open-label VEN was started in Phase 1 at an initial dosage of 37.5 mg *p.o.*, with a target dose of 225 mg *q.d.* by the end of Phase 1. This dosage was continued following randomization in Phase 2. Open-label Li was started at 300 mg/day on the day of randomization in Phase 2, with a target level for most patients in the 0.4-0.6 mEq/L range. For

VEN and Li dosing/procedures were identical for the PHARM arm and the STABLE + PHARM arm, except that Li was withheld the night before ECT in the STABLE + PHARM arm. The schedule of clinic and telephone ratings was identical for both the PHARM and STABLE + PHARM arms.

Assessments

HRQOL

HRQOL was measured with the Medical Outcomes Study Short Form 36 (SF-36) (J. E. Ware, 2003; J. E. Ware, Jr. & Sherbourne, 1992). The SF-36 was measured at baseline prior to acute ECT and again at the end of Phase 1/beginning of Phase 2. Thereafter, the SF-36 was measured every 4 weeks during Phase 2. SF-36 data were scored in terms of the 8 standard subscales: Physical Functioning (PF), Role Physical (RP), Bodily Pain (BP), General Health (GH), Vitality (VT), Social Functioning (SF), Role Emotional (RE), and Mental Health (MH). The score for each subscale is the weighted sum of the questions for that subscale, transformed into a 0-100 scale. Lower scores define more disability. Individual scores were then transformed into T-scores, with means of 50 and standard deviations of 10. The 8 subscales were then aggregated into the two total scores: Physical Health Factor T-score (comprised of PF, RP, BP, and GH subscales) and Mental Health Factor T-score (comprised of the VT, SF, RE, and MH subscales) following the algorithm provided by Ware, Kosinski & Dewey, by weighing the 8 subscale scores by their respective physical and mental factor scores and summing over the physical and mental subscale scores (J. E. Ware, Kosinski, & Dewey, 2000). In addition, there are two age- and sex-adjusted summary scores; physical component score (PCS) and mental component score (MCS).

Depression severity and remission/relapse status

Depressive symptom severity was measured using the HRSD₂₄, and was treated as a continuous variable for the purposes of the relationship between HRQOL and depression severity

(Hamilton, 1960). However, participants left Phase 2 of the study if they relapsed, which was defined as two consecutive HRSD₂₄ scores ≥ 21 , or the need for psychiatric hospitalization, or becoming suicidal (Kellner, Husain, Knapp, McCall, Petrides, Rudorfer, Young, Sampson, McClintock, Mueller, Prudic, Greenberg, Weiner, Bailine, Rosenquist, Raza, Kaliora, Latoussakis, Tobias, Briggs, Liebman, Geduldig, Teklehaimanot, Dooley, et al., 2016).

Cognitive Function

The Wechsler Test of Adult Reading (WTAR) was administered once at Phase 1 baseline as an estimate of pre-morbid intellectual functioning (The Psychological Corporation, 2001). Global cognitive function was measured with the Mini Mental State Examination (MMSE)(Folstein, Folstein, & McHugh, 1975).

The second edition of the California Verbal Learning Test (CVLT-II) was used to assess delayed recall of verbal information, expressed as “% retention” (Delis, Kaplan, Kramer, & Ober, 2000; Woods, Delis, Scott, Kramer, & Holdnack, 2006).

The Dementia Rating Scale-2nd edition Initiation/Perseveration Index (DRS-2 I/P) was used to measure executive function (Lezak, 2004). The MMSE was measured every two weeks, CVLT-II monthly, and DRS-2 I/P was measured at midpoint and end of the study period.

Randomization and Masking of Treatment Assignment

The permuted block method of randomization was used, stratified by site. Block size was varied to minimize the likelihood of unmasking. Clinical raters and neuropsychological technicians were masked to treatment assignment.

Statistical Analysis

All statistical analyses were done by the authors (RGK, MM, and MD). Analysis was conducted on an intent-to-treat (ITT) sample using SAS 9.4. Longitudinal analyses were conducted using mixed effect models (MEM) to examine change in SF-36 sub-scores over a 24-week period. Simple models were analyzed for both the 2 SF-36 component and total scores, as well as the 8 sub-scores, with the total scores/subscores as the dependent variable and with fixed effects for the independent variables treatment (STABLE vs. PHARM), time (even weeks), and the time-by-treatment interaction. Expanded models adjusted the simple models for clinical site as a stratification variable, psychosis, WTAR, and time varying effect of CVLT-II retention, DRS-2 I/P, and MMSE. All available data for these variables collected during Phase 2 were used in these models. Treatment interactions for age and psychosis were tested for significance. Additionally, expanded models for the total scores and sub-scores were adjusted for age and gender, whereas the PCS and the MCS were calculated as age- and gender-adjusted scores and therefore did not need further adjustment. Furthermore, expanded models were adjusted for the time varying effect of HRSD₂₄, except for those sub-scores that included questions that were similar to those

of HRSD₂₄ (MCS, Mental Health Factor T-Score, and MH). To account for random effects of subjects, random intercepts were used in all models and random slopes were used in models where the G-matrix was positive definite.

RESULTS

120 patients were randomized to PHARM (N=59) and STABLE + PHARM (N=61). Patients entering into Phase 2 had a mean HRSD₂₄ total score of 6.1; the majority were female (61.7%) and white (95%), with a mean age of 70.5 ± 7.2 years. The patients assigned to STABLE + PHARM received an average of 4.5 ± 2.5 continuation ECT sessions during Phase 2.

The time and group adjusted SF-36 mean score difference at 24 weeks was statistically significant between PHARM and STABLE + PHARM for PCS, MCS, total scores and all of the sub-scores (all $p < 0.02$) except the BP sub-score ($p = 0.078$). Patients randomized to STABLE + PHARM had significantly higher quality of life scores at the 24-week visit compared to patients in the PHARM group. All SF-36 sub-scores as well as PCS and MCS showed a statistically significant time-by-treatment interaction in the simple models (all $p < 0.04$, Table 1) indicating that the effect of time on quality of life depended on the treatment arm.

When the simple models were adjusted for additional covariates, all models except for the SF-36 sub-scores SF, RP, and RE resulted in statistically significant adjusted mean sub-score differences at 24 weeks (all $p < 0.04$, results not shown). HRSD₂₄ was statistically significantly associated with SF-36 PCS and sub-scores in all models where it was included except for Physical Health Factor T-Score (Table 2). Group assignment to STABLE + PHARM versus PHARM did not significantly contribute to the model after accounting for the effects of HRSD₂₄ (Table 2). For models that did not include HRSD₂₄, time was statistically significantly associated with both SF-36 MCS and Mental Health Factor T Score (all $p < 0.02$) but not MH sub-score ($p = 0.09$). Age was statistically significantly associated with SF-36 sub-scores Physical Health Factor T-Score, PF, and RP (Table 2). Of particular note, cognitive variables had modest associations to HRQOL variables (Table 2).

DISCUSSION

This is the first report of the HRQOL effects in a randomized comparison of a treatment strategy that included ECT combined with medication versus medication alone. Participants who received ECT in the STABLE + PHARM group had better HRQOL on every dimension of the SF-36 across 6-months of follow-up compared with the patients in the PHARM group. The HRQOL benefits were best explained by superior control of depressive symptoms in the STABLE + PHARM group, with negligible contribution of cognitive function variables. The results of this study are especially meaningful since elderly participants with severe MDD may be viewed as a particularly vulnerable population who are referred for ECT (W. V. McCall et al., 1999; McCall & Dunn, 2003). These results provide strong evidence that ECT produces an overall net health benefit. Strengths of this study included a well-controlled design, use of standardized clinical neuropsychological variables to assess cognitive function, use of standardized psychometrically sound measures of

HRQOL, and an accounting of other confounding variables. However, as this study was designed to examine prevention of depressive relapse and was not designed to examine acute antidepressant effects, caution is warranted in drawing conclusions about HRQOL effects of ECT during acute treatment of depression in which the ECT treatments would be given more frequently than was done in this relapse prevention intervention.

The current report has some limitations. First, the measure of HRQOL was completed by patient self-report, and we did not include any measures of clinician-rated or third-party observers, but in prior work we showed that ECT patients' assessment of their functional status closely matched the assessments of observers (McCall, Dunn, Rosenquist, & Hughes, 2002). Second, lithium, venlafaxine, and as-needed lorazepam were the only medications examined in this study, while RUL UB ECT was the only form of ECT. Other medication regimens and other ECT treatment approaches may have produced different results. Indeed, different electrode placements have been shown to have differential effects on HRQOL, with RUL having superior HRQOL outcomes as compared with bilateral electrode placement (Galvez et al., 2016; McCall et al., 2011). Finally, the number of ECT treatments in the STABLE arm was low in comparison to a typical acute course of ECT; however, it is remarkable that a low number of ECTs were sufficient to enhance HRQOL over a 6-month period.

The present report adds to the literature on the beneficial effects of ECT on HRQOL and the overall net health benefits. Our findings should assure patients, families and caregivers of depressed elderly patients that ECT is a medically appropriate choice for elderly adults with MDD and is likely to produce improvement in HRQOL that can be sustained during the continuation phase of treatment by employing combined ECT and medication to support remission. The results of the PRIDE study support the merit of continuing ECT as a means of staving off depressive symptoms. Our findings substantiate that for elderly adults with MDD, ECT can be a quality-of-life-enhancing treatment when used as a continuation strategy in combination with medication to prolong remission.

Table 1
 “Basic Model SF-36 Total and Subscales at week-24, by Treatment adjusted for Time, and Treatment-by-Time interaction”.

SF-36 T-SCORE	Treatment*Time	Week 24 adjusted				BASELINE						
	p-value	PHARM	STABLE + PHARM		DIFFERENCE	PHARM	STABLE + PHARM		DIFFERENCE			
		Mean	(SE)	Mean	(SE)	Mean (95% CI)	p-value	Mean	(SE)	Mean	(SE)	Mean (95% CI)
PHYSICAL												
Physical Component Score (PCS) ^a	< 0.0001	44.4	(1.2)	51.3	(1.2)	-7.0 (-10.4, -3.5)	0.0001	45.3	(0.8)	44.5	(1.1)	0.8 (-1.8,3.4)
Physical Health Factor T Score	0.0127	44.1	(1.4)	49.0	(1.3)	-4.9 (-8.7, -1.1)	0.0125	49.1	(1.2)	48.5	(1.4)	0.7 (-3.0,4.3)
Bodily Pain (BP)	0.0361	48.4	(1.5)	52.1	(1.4)	-3.7 (-7.9, 0.4)	0.0777	49.4	(1.2)	48.4	(1.5)	1.1 (-2.8,4.9)
General Health (GH)	0.0006	43.9	(1.6)	52.3	(1.5)	-8.4 (-12.7, -4.1)	0.0002	48.8	(1.0)	49.3	(1.0)	-0.5 (-3.3,2.3)
Role Physical (RP)	< 0.0001	40.9	(1.5)	49.6	(1.4)	-8.7 (-12.7, -4.6)	< 0.0001	42.3	(1.2)	39.2	(1.5)	3.1 (-0.8,6.9)
Physical Functioning (PF)	0.0057	41.0	(1.6)	46.7	(1.6)	-5.7 (-10.1, -1.2)	0.0133	43.2	(1.3)	42.4	(1.7)	0.9 (-3.2,5.1)
MENTAL												
Mental Component Score (MCS) ^a	< 0.0001	45.3	(1.5)	53.5	(1.4)	-8.2 (-12.2, -4.2)	0.0001	41.3	(0.9)	40.5	(1.0)	0.8 (-1.9,3.6)
Mental Health Factor T Score	0.0292	47.6	(2.0)	54.4	(1.9)	-6.7 (-12.3, -1.2)	0.0174	35.6	(1.5)	34.5	(1.5)	1.1 (-3.2,5.3)
Mental Health (MH)	0.0086	47.0	(1.6)	54.2	(1.6)	-7.2 (-11.6, -2.7)	0.0017	39.7	(1.2)	37.4	(1.2)	2.3 (-1.1,5.7)
Role Emotional (RE)	0.0006	42.8	(1.7)	50.4	(1.6)	-7.6 (-12.3, -3.0)	0.0016	35.0	(1.4)	32.1	(1.6)	2.9 (-1.3,7.1)
Vitality (VT)	0.0108	47.8	(1.7)	54.5	(1.7)	-6.7 (-11.4, -2.0)	0.0061	44.3	(1.1)	45.0	(1.2)	-0.6 (-3.8,2.5)
Social Functioning (SF)	0.0051	44.7	(1.5)	53.3	(1.4)	-8.6 (-12.7, -4.5)	< 0.0001	35.1	(1.4)	36.4	(1.4)	-1.2 (-5.2,2.7)

^a Subscale scores are age and gender adjusted; higher scores are better.

Table 2
 “Regression coefficients for predictors of SF-36 Total and Subscales over 24 weeks for STABLE + PHARM?”

OUTCOME	β -coefficients									
SF-36 T-SCORE	STABLE + PHARM	HRSD	MMSE	WTAR	% Retention	DRS	Psychosis	Age	Female	
Physical Factors										
Physical Component Score (PCS) ^a	1.90	-0.63*****	-0.05	0.09	0.01	-0.13	-0.15	NA ^a	NA ^a	
Physical Health Factor T Score	1.76	-0.09	-0.06	0.10	0.02	-0.13	3.91	-0.30*	-3.07	
Bodily Pain (BP)	2.14	-0.42**	0.02	0.07	0.02	-0.04	4.50	-0.27	0.04	
General Health (GH)	3.20	-0.57*****	0.02	-0.10	0.04	-0.28	-1.53	-0.04	0.87	
Role Physical (RP)	2.88	-0.67*****	-0.03	0.23*	-0.02	0.07	2.09	-0.42**	-1.98	
Physical Functioning (PF)	1.15	-0.29*	-0.03	0.24*	0.04	-0.39	-1.48	-0.39*	-5.77***	
Mental Factors										
Mental Component Score (MCS) ^a	1.23	NA ^b	-0.05	-0.02	0.01	0.05	2.88	NA ^a	NA ^a	
Mental Health Factor T Score	-1.62	NA ^b	-0.03	-0.08	-0.02	0.01	5.99	0.32	-2.17	
Mental Health (MH)	2.89	NA ^b	-0.10	-0.10	0.04	0.38	5.29	0.33	-3.87	
Role Emotional (RE)	1.21	-1.23*****	0.02	0.15	-0.03	-0.10	-5.39	-0.15	-0.27	
Vitality (VT)	2.95	-1.23*****	0.04	0.08	-0.04	-0.23	2.13	-0.14	-0.93	
Social Functioning (SF)	2.34	-1.05*****	0.08	0.11	-0.01	-0.34	1.04	-0.22	0.49	

*p-value < 0.05; ** p-value < 0.01, *** p-value < 0.001, ****p-value < 0.0001.

SF-36: Short-Form 36.

STABLE: Symptom-Titrated, Algorithm-Based Longitudinal ECT.

HRSD: 24-item Hamilton rating Scale for Depression.

MMSE: Mini Mental State Examination.

WTAR: Wechsler Test of Adult Reading.

% Retention: delayed recall from the second edition of the California Verbal Learning Test (CVLT-II).

DRS: The Dementia Rating Scale-2nd Edition Initiation/Perseveration Index.

^a Subscale scores are age and gender adjusted.

^b Subscale scores included questions that were similar to those of HRSD₂₄ therefore excluded HRSD₂₄ from model.

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Chapter 10 Charting The ECT Course: Where Have We Been? Where Are We Headed?

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Abstract

Electroconvulsive Therapy (ECT) is one of the oldest and arguably remains the gold standard treatment for severe mental illness in psychiatry. A safe and highly effective option for treatment-resistant mood disorders, ECT is positively life-saving for those suffering from catatonia and acute suicidality. Less recognized are ECT's benefits in the treatment of primary psychotic disorders, Parkinson's disease and status epilepticus. In the past decade, evidence from multi-site clinical trials highlights an evolving standard for the delivery of ECT to achieve and maintain remission and quality of life. Today, the optimal practice of ECT is defined by evidence-based treatment planning including patient selection, choice of electrode placement and stimulus parameters, augmentation with pharmacotherapy, and the use of continuation/maintenance treatment. Research into biomarkers and neuroplasticity related to ECT response, as well as new, investigational methods of delivering ECT provide a glimpse into the future of this time-honored treatment.

INTRODUCTION

ECT for severe mental illness has withstood the test of time, despite persistent stigma based upon the perpetuation of an inaccurate and antiquated view of the procedure. Patients and caregivers ask, “Shock therapy is still being used today?” The answer is definitively yes—but today’s ECT is dynamic and steadily improving. In this review, we trace changes in technique emerging from treatment-oriented research through the past several decades. Even as this work has produced demonstrable improvements, many unanswered questions point the way forward.

PATIENT SELECTION AND OUTCOME

Every treatment may be characterized by range of efficacy, durability, dose-response and dose-toxicity relationships. Patient selection is the first principle of optimization. Remarkably broad spectrum in range of efficacy, strong evidence supports the use of ECT for mood disorders—both major depressive (MDD) and bipolar disorder (Bailine et al., 2010). Catatonia, now recognized in DSM-5 as a separate syndrome occurring in conjunction with a number of other manifestations of brain pathology, is one indication for which ECT may be uniquely beneficial (Luchini et al., 2015). Similarly, ECT should be considered “first-line” in the treatment of mood disorders with psychotic features because of its rapid onset and effectiveness (Petrides et al., 2001). Other presentations for which ECT is appropriately elevated in the treatment algorithm include acute/high-risk suicidal ideation and behavior (Fink, Kellner, & McCall, 2014) late-life depression (Dombrovski & Mulsant, 2007), and Parkinson-plus syndromes where ECT has demonstrated benefits on motor and non-motor symptoms (Narang, Glowacki, & Lippmann, 2015). While still seen as “last resort,” ECT successfully aborts treatment-resistant status epilepticus in up to 80% of reported cases (Lambrecq et al., 2012).

Less recognized are the benefits in the treatment of primary psychotic disorders as well as disturbances of mood and thought resulting from neurologic or substance-related causes. A curious divergence in the worldwide use of ECT has occurred with schizophrenia emerging as the primary indication for ECT in Asia, as opposed to mood disorders in the West. While the reasons for this are not entirely clear, the evidence base has lagged somewhat for psychotic disorders. Recent work has demonstrated additive effects of antipsychotic medication therapy when combined with ECT in treatment-resistant schizophrenia (Wang et al., 2015). Of those dimensions of psychotic disorders currently identified in DSM-5, ECT appears to be most beneficial for catatonic, positive and mood symptoms, and to a lesser extent cognition, negative symptoms, and thought disorder, although the mechanism of its broad spectrum of effects is less clear (Rosenquist, Miller, & Pillai, 2014).

While treatment resistance often results in referral for ECT, it should be understood that this may diminish expectation of efficacy for MDD (Haq, Sitzmann, Goldman, Maixner, & Mickey, 2015). Nevertheless, patients who have failed to respond to multiple medication trials may be more likely to benefit from ECT compared with another round of medication. The presence of a comorbid personality disorder appears to have a negative influence on the outcome of ECT as does duration of illness, suggesting that ECT is most beneficial for more acute and/or episodic neuropathological states (Rasmussen, 2015; Rasmussen, Hart, & Lineberry, 2008). However, ECT

practitioners frequently encounter treatment-refractory patients for whom the administration of ECT results in symptomatic improvement whereupon other forms of treatment including psychotherapy became more viable.

The critics of ECT have charged that ECT is associated with a net loss of health, but the literature on quality of life (QOL) and function presents a very different picture. QOL measures dimensions such as freedom from pain, ability to enjoy relationships, managing one's own life, etc. Patients with major depressive disorder (MDD) have poor QOL compared with ambulatory primary care patients without MDD; those referred for ECT have even worse QOL. ECT confers immediate improvements in mean QOL and function in 80-90% of MDD patients, which are sustained for 6 months (McCall et al., 2013). Importantly, the improvement in QOL is explained by improvements in mood, while the patient's cognitive status after ECT does not contribute to the variance in QOL. ECT is a safe procedure, but increasing age and preexistent cardiac disease increase the risk of minor and major complications. A recent veteran's affairs study showed no deaths associated with ECT in any VA hospital between 1999 and 2010. Based on the number of treatments given, the authors estimate the mortality rate associated with ECT as less than 1 death per 73,440 treatments, with the most common adverse events reported being injury to the mouth (including dental and tongue injury) and problems related to paralysis (Watts, Groft, Bagian, & Mills, 2011).

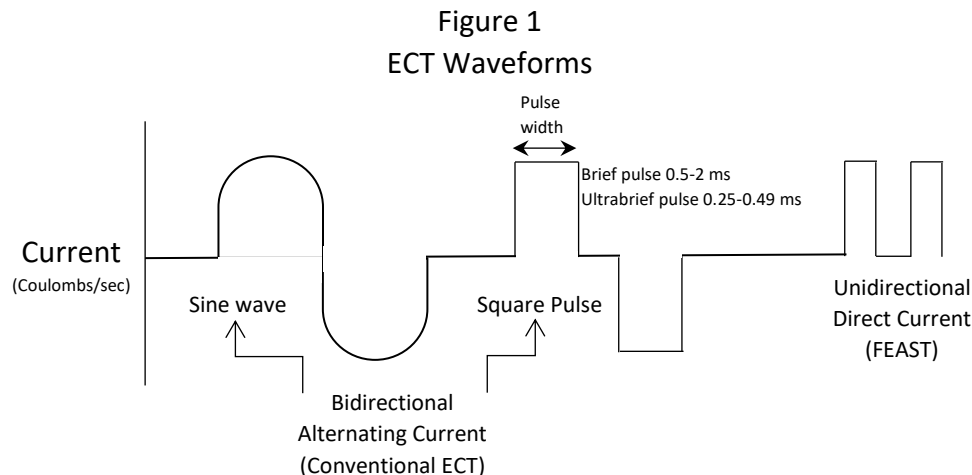
TREATMENT COURSE

The question frequently arises, "how many ECT sessions are required?" A standard answer might be 6-12 treatments in a so-called "index" acute course administered 2-3 times weekly until the patient remits, defined by a rating scale score using a clinician or patient self-rated instrument validated for this purpose (Hamilton, 1960; Rush et al., 2006). The use of such measures ensures that patient and provider are cognizant of gradual improvements which lag behind global assessments prejudiced by depressive cognitive bias. Speed of response is variable, with some patients noting rapid cessation of suicide ideation or achieving full remission within a few treatments. Longer treatment courses are generally expected in order to achieve meaningful improvements in symptoms of schizophrenia.

DOSING

One might ask why not optimize treatment by using the highest dosage from the outset of treatment? Like drug treatment, ECT is associated with dose-dependent cognitive side effects—post-ictal impairments in attention, concentration and deficits in autobiographical memory. These develop over the course of treatment and can often be mitigated by a step-wise approach. Many practitioners have adopted a method of dose-titration, thereby identifying an individualized seizure threshold defined by the lowest electrical charge required to initiate a seizure in a particular patient. This forms the basis for dosing subsequent treatment. Typically, bitemporal (BT) and bifrontal (BF) treatment is delivered at 1.5 to 2 times the seizure threshold, whereas right unilateral (RUL) treatment requires 4-6 times the seizure threshold in order to achieve a positive treatment outcome (Kellner et al., 2010).

Figure. Electrode Placement and Stimulus Characteristics



Initial choice of electrode placement is subject to some interpretation. Compared with RUL and BF, BT ECT is associated with significantly greater risk of cognitive impairment (Dunne & McLoughlin, 2012; Sackeim et al., 2007). Yet, many ECT practitioners default to BT treatments based upon the perception of more robust or more rapid response. However, the body of evidence in support of RUL ECT suggests that crossover to BT is required in a minority of cases.

The nature of the ECT stimulus has evolved over time (Figure 1). Though effective at seizure generation, sine wave ECT was shown to be definitively more cognitively impairing and is now considered outdated (Sackeim et al., 2007). Modern ECT devices deliver a rectified “square” pulse. The square pulse is more efficient and therefore requires less total charge to induce a seizure. Similarly, decreasing the pulse width (2 milliseconds versus 1 millisecond) is more efficient and less likely to produce cognitive impairment. “Brief” pulses of 0.5 to 1 ms are generally effective and well tolerated. More recently “ultra-brief” pulse ECT defined as less than 0.5 ms (commonly 0.25 to .37 ms with current devices) have appeared to be still more efficient and cognitively benign. For unclear reasons, the initial study of ultra-brief stimuli, ultra-brief BT ECT was less effective than all other arms in the study (brief BT, brief and ultra-brief RUL) (Sackeim et al., 2008). While confirming the relative cognitive advantage of ultra-brief RUL, other studies have produced conflicting results about efficacy. Some studies show lower rates of remission or prolonged treatment course, but efficacy appears to be comparable at a higher stimulus intensity (8 times seizure threshold) (Loo et al., 2014).

COORDINATION WITH OTHER THERAPIES TO ACHIEVE AND MAINTAIN REMISSION

ECT is generally not employed as monotherapy. One important discovery was that the remission rate for MDD is improved with concomitant antidepressant medication therapy. ECT plus placebo resulted in a remission rate of only 49%, whereas the addition of venlafaxine or

nortriptyline improved this to 62% and 64% respectively (Sackeim et al., 2009). A lack of antidepressant treatment following remission with ECT results in relapse for 90% of patients, whereas maintenance pharmacotherapy with antidepressant alone is only marginally effective (61% relapse within 6 months) (Sackeim et al., 2001). Augmentation of antidepressants with lithium is the gold standard for pharmacotherapy following ECT, reducing relapse rates to only 2-40% (Nordenskjold et al., 2013; Sackeim et al., 2001).

There is emerging evidence that the addition of maintenance ECT might be more helpful in treatment-resistant cases to maximize chances of sustaining remission (Youssef & McCall, 2014). In a large randomized trial, maintenance ECT alone was demonstrated to be equivalent to lithium plus nortriptyline (sustained remission rate of 46%) (Kellner et al., 2006). Additional studies have shown that maintenance ECT plus pharmacotherapy is more effective than pharmacotherapy alone at one and two year follow-up (Navarro et al., 2008).

BIOMARKERS

There are no widely accepted biomarkers of clinical response, though a number of studies have shown that electroencephalographic measures of induced seizure duration or morphology are modest predictors. These so-called “seizure quality” measures may be identified by visual inspection or by algorithms built into the ECT device. When the patient is improving as expected, such measures are of little consequence. However, when the patient is slow to respond, seizure quality measures may provide additional impetus to increase stimulus dose, change electrode placement or withdraw concomitant medications in order to potentially improve the seizure quality.

Preliminary studies in humans and animals show favorable effect of ECT in inducing neurogenesis and neuroplasticity. One study demonstrated increased hippocampal and amygdala volumes after ECT which correlated with an improved clinical response (Joshi et al., 2016). These data have attracted attention to the possible role of adult neurogenesis in the antidepressant effect of pharmacological agents (Rotheneichner et al., 2014).

THE FUTURE OF ECT

The future of ECT depends upon continued refinement of the technique to minimize side effect burden while maintaining effectiveness. Two lines of innovation are currently being investigated with the goal of diminishing side effects by making seizure activity more focal—magnetic seizure therapy (MST) and focal electrically-administered seizure therapy (FEAST). MST capitalizes on the fact that the skull is transparent to the magnetic field and easily overcomes scalp/skull impedance. The field is transferred in the brain by Eddy currents (Faraday's law of induction) with more focality (Lisanby, Luber, Schlaepfer, & Sackeim, 2003). FEAST uses a unidirectional-pulsed stimulus along with asymmetrically-sized electrodes (Nahas et al., 2013) to deliver a focal current to the target areas believed to be most responsible for depression, including the right orbitofrontal cortex.

Preliminary research suggests that FEAST and MST are cognition-sparing, compared to traditional ECT but have yet to demonstrate comparable efficacy. MST presents unique engineering challenges because of inefficient energy transfer to brain tissue requiring excessively high-powered devices to generate a seizure. Also, the penetration in the brain is superficial with a depth of only 2-4 cm below the coil (Deng, Lisanby, & Peterchev, 2011).

While we envision that ECT will remain the backbone of treatment for severe and treatment-resistant psychiatric illness, mounting experience with alternative brain stimulation modalities such as repetitive transcranial magnetic stimulation, and transcranial direct current stimulation may well provide novel insights to guide the further development of this time-honored treatment.

Contributions on this manuscript for the purpose of this thesis: Nagy Youssef searched the literature, wrote about a third of the paper, as the coauthors divided the sections to be written, and all coauthors edited the interim versions of the paper as well as the final paper. Other authors did similar effort and wrote the rest of the manuscript. In addition, Peter Rosenquist, the first author, packaged the final manuscript and responded to reviewers' suggestions.

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Chapter 11 The Role of ECT in Posttraumatic Stress Disorder: A Systematic Review

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Abstract

Background: Posttraumatic stress disorder (PTSD) is associated with a high burden of disability and mortality and frequently is treatment-resistant. There is little to offer patients who are not responding to standard interventions. Thus, the objective of this report is to systematically review human data on whether electroconvulsive therapy (ECT) is effective in PTSD.

Methods: We performed a systematic literature review through August 2016 for clinical studies and case reports published in English examining the efficacy of ECT in improving PTSD symptoms.

Results: The literature search generated 3 retrospective studies, 1 prospective uncontrolled clinical trial, and 5 case reports. It is not clear given the small sample size and lack of a large randomized trial whether favorable outcomes were attributed to improvement in depression (as opposed to core PTSD symptoms).

Conclusions: Current efficacy data do not separate conclusively the effects of ECT on PTSD symptoms from those on depression. Randomized controlled trials are therefore necessary to examine the use of ECT in medication-refractory PTSD patients with and without comorbid depression. Subsequent studies may address response in PTSD subtypes, and the use of novel techniques such as memory reactivation before ECT.

INTRODUCTION

The lifetime prevalence of posttraumatic stress disorder (PTSD) in the United States is approximately 7.8% (R. C. Kessler, Chiu, Demler, Merikangas, & Walters, 2005). The lifetime prevalence in other countries (using different survey administration and sampling strategies) for PTSD ranges from 0.3% in China to 6.1% in New Zealand (R. Kessler & Ustun, 2008). PTSD is associated commonly with a high burden of disability, high suicide risk (Davidson, Hughes, Blazer, & George, 1991) and functional impairment (Amaya-Jackson et al., 1999; Thomas et al., 2010). If not treated adequately, the prognosis often is chronic (R. C. Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995).

PTSD is frequently associated with other psychiatric comorbidities, which further complicates treatment strategies and response to treatment. This includes 45% comorbidity with major depression and more for depressive symptoms that are subsyndromal (Shalev et al., 1998). Suicidality (Davidson et al., 1991; Guerra, Calhoun, & Mid-Atlantic Mental Illness Research, 2011) and substance use disorders (R. C. Kessler et al., 2005; Nock, Hwang, Sampson, & Kessler, 2010; Wilcox, Storr, & Breslau, 2009) further complicate PTSD (Dedert et al., 2009).

Individuals with PTSD also have higher rates of other physical disorders (Davidson et al., 1991) compared to those without PTSD in a community sample. Thus, those with PTSD have higher rates of mental and physical health services compared to those without PTSD (Calhoun, Bosworth, Grambow, Dudley, & Beckham, 2002).

The first-line pharmacological treatment for PTSD is selective serotonin reuptake inhibitors (SSRIs). Nonetheless, less than one-half of patients achieve full remission (Amiel, Mangurian, Ganguli, & Newcomer, 2008; Berger et al., 2009). Even fewer achieve full remission for combat-related PTSD and chronic PTSD (Davidson et al., 2003; Hertzberg, Feldman, Beckham, Kudler, & Davidson, 2000). In addition, the effect size of improvement from SSRIs (standardized mean difference -0.23 , 95% CI -0.33 to -0.12) and pharmacotherapy (including studies of monoamine oxidase inhibitor, other antidepressants, olanzapine, and topiramate) in general has been found to be small according to a well-done meta-analysis (Hoskins et al., 2015).

Currently, sertraline (Brady et al., 2000; Schwartz & Rothbaum, 2002) and paroxetine (Marshall et al., 2007; Tucker et al., 2001) are FDA-approved for PTSD. Nonetheless, many patients are unresponsive to first-line therapy with SSRI (Youssef et al., 2012). Also, SSRI trials have found that more than 50% of patients have incapacitating residual symptoms. A well-designed trial of prazosin in active duty soldiers with PTSD found significant improvement in PTSD symptoms, especially nightmares, and sleep quality using prazosin. Although patients responded to prazosin, PTSD symptoms did not remit (mean Clinical-Administrated PTSD Scale (CAPS) score dropped from 77 to 52), and not all patients responded (Raskind et al., 2013). The effects of prazosin also have been demonstrated in civilian patients with PTSD (Taylor et al., 2008).

Pharmacotherapy and trauma-oriented psychotherapy (Cusack et al., 2016) are the most common treatment modalities used in clinical practice. Although these two modalities are mechanistically different and involve a multidisciplinary team, only approximately 50% of patients have remission of symptoms with both modalities. The other approximately 50% of patients remain treatment-resistant (R. C. Kessler et al., 1995).

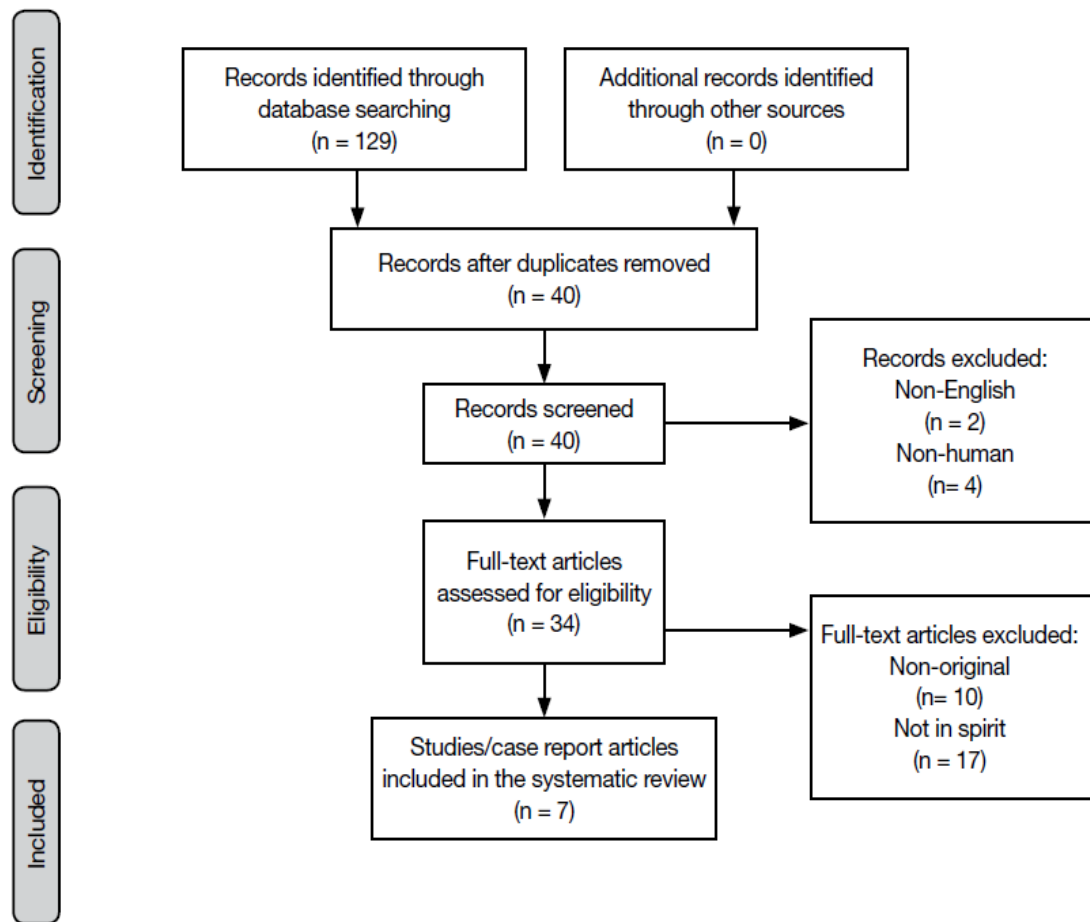
Electroconvulsive therapy (ECT) is the most effective treatment for major depressive (MDD) and bipolar disorders. Furthermore, it is also the most effective treatment for depressed patients who are treatment-resistant to pharmacotherapy and psychotherapy (Lisanby, 2007). However, PTSD is not a recognized indication for ECT. In this manuscript, we systematically review the available evidence in order to evaluate the effectiveness of ECT in treating PTSD, especially severe or treatment-resistant forms.

METHODS

We reviewed the medical literature for papers published on ECT treatment of patients with PTSD. We included reports in English from 1958 to August 2016 using PubMed/Medline. We also searched the Web of Science database and the Clinicaltrials.gov registry. We used the search terms: electroconvulsive therapy and posttraumatic stress disorder; electroconvulsive therapy and PTSD; ECT and PTSD. In PubMed, we also added the terms "Stress Disorders, Post-Traumatic"[Mesh] AND "Electroconvulsive Therapy"[Mesh]. We also searched the bibliographies of the found articles and contacted several experts in the field for any possible additional publications/studies. As expected, there was overlap between the above searches. All papers were found in both PubMed and Web of Science except for Ozdemir et al.,(Ozdemir, Garip, Oznur, Erdem, & Akarsu, 2013) which was only found in the Web of Science. Only one clinical trial was found in Clinicaltrials.gov registry, by Margoob et al.(Margoob, Ali, & Andrade, 2010). See Figure 1 for PRISMA diagram.

FIGURE

PRISMA diagram for the systematic literature review of original reports on the use of electroconvulsive therapy in patients with posttraumatic stress disorder



Source: Moher D, Liberati A, Tetzlaff J, et al; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med*. 2009;151(4):264-269, W64.

RESULTS

The literature search generated 7 published papers relevant to the efficacy of ECT in PTSD. There were no relevant papers published in languages other than English. Our findings included 3 studies (Table 1) and 5 case reports published in 4 papers (Table 2).

Studies of PTSD

In 2007, Watts (Watts, 2007) conducted a retrospective chart review study to examine the use of ECT for treatment of PTSD in 26 military veterans with PTSD and comorbid major depression. The study was carried out in a VA Hospital in the New England area (from January of 1998 to December of 2002).

Out of 77 patients who were treated with an index course of ECT, 28 patients met the criterion of both current MDD and PTSD according to DSM-IV. Of those, 26 patients had complete baseline and endpoint assessments using PTSD Checklist (PCL)(Forbes, Creamer, & Biddle, 2001) and the Montgomery-Åsberg Depression Rating Scale (MADRS)(Montgomery & Asberg, 1979). These 26 patients were included in the statistical analysis. Sixteen patients started and completed the acute course of ECT while hospitalized, while 10 patients completed the course after hospital discharge.

Routine psychiatric medications were tapered and discontinued before ECT (as is usually the practice at that center). ECT was administered using MECTA SpECTrum 5000Q device (MECTA Corporation, Lake Oswego, OR). Anesthesia was administered with methohexital, and the muscle relaxant was succinylcholine. All patients started treatment with right unilateral ECT (administered at 2.5 times the seizure threshold). Twenty-one patients received unilateral treatments only (unilateral treatments sessions 9.7 [SD, 1.6]). Five patients who were non-responders to right unilateral (RUL) ECT were switched after 8 treatments to bitemporal ECT (bitemporal treatments 4.6 [SD, 1.2]).

PTSD response was defined as $\geq 20\%$ reduction in PTSD symptoms on the PCL. Depression response was defined as a 50% reduction in depressive symptoms, and remission was defined as $\geq 60\%$ reduction plus final MADRS of ≤ 10 . The statistical analysis used a paired t-test comparing pre-treatment to post-treatment level of symptoms on the PCL and MADRS.

This study found that patients receiving ECT had a significant reduction in the symptoms of major depression and a statistically insignificant reduction in PTSD symptoms (Table 1). The authors concluded that “electroconvulsive therapy may be an effective treatment for patients with refractory depression and co-occurring PTSD.” However, the major limitations were the small sample and the retrospective chart review nature of the study, which may have affected the accuracy of the PTSD diagnosis retrospectively.

A prospective open-label study by Margoob and Andrade (Margoob et al., 2010) enrolled 20 consecutive adult civilian patients with severe, chronic, treatment-resistant PTSD. Treatment resistance was defined as failure to respond to an adequate trial of at least 4 antidepressant medications and 12 sessions of cognitive-behavior therapy. Participants were treated with a fixed index course of 6 bilateral ECT sessions (sessions administered outpatient twice-weekly). Anesthesia was administered with pentothal; the muscle relaxant was succinylcholine. ECT was administered using the MECTA-SR1 device with current amplitude of 800 mA, pulse width of 1.5 ms, and frequency of 50 Hz. Stimulus duration was increased as needed to elicit an adequate seizure. An adequate seizure was defined as an EEG seizure of least 25s. The stimulus intensity was barely suprathreshold. Psychotropic medications were tapered and stopped before the ECT course.

CAPS was used as the primary outcome measure. Response was defined as at least a 30% decrease in CAPS, and remission as a CAPS score of < 20 . Secondary outcome measures were the MADRS and the Clinical Global Impression-Severity scale (CGI-S).

Out of 20 participants, 17 completed the ECT course. An intent-to-treat analysis (N=20) showed a decrease in the CAPS scores by 34% ($P < .001$), and MADRS scores decreased by 51.1%

($P < .001$). The authors reported that most of the improvement in PTSD and depression ratings developed by the third ECT session. It also was noted that the improvements in CAPS were independent of the improvements in MADRS. The authors noted that “improvement in CAPS did not differ significantly between patients with less severe vs more severe baseline depression”. Statistical tests included paired t -test used to compare pre-post time points (before ECT, and after the sixth ECT session) and repeated measures ANOVA to compare across the three time points (before ECT, after the third, and the sixth ECT sessions).

A complete analysis also was performed for the 17 participants who completed the study. In those participants, CAPS scores dropped from 92 to 55 (40%). The MADRS scores dropped from 24 to 10 (57%). The CAPS response rate occurred in 82% of participants. The correlation between improvement in CAPS and improvement in MADRS was low and was not statistically significant.

Four of these patients had psychotic symptoms at baseline. In those patients, CAPS scores dropped from 86.5 to 47.0 (46%). The MADRS scores dropped from 23.3 to 9.5 (59%). The psychotic symptoms remitted in 3 patients (and were reduced markedly in the 4th). Treatment response (assessed clinically, not by rating scales) was maintained at a 4 to 6 month follow-up.

This was the only study in which PTSD was the indication for ECT (rather than depression or a psychotic disorder with comorbid PTSD in the other reports). On the other hand, patients were not formally assessed for comorbid MDD, and so the prevalence of MDD in the sample was not known.

In a retrospective, nested, matched case-control study, Ahmadi et al. (Ahmadi, Moss, Simon, Nemeroff, & Atre-Vaidya, 2015) compared consecutive patients with PTSD and comorbid MDD who received ECT ($N=92$) to those with PTSD and comorbid MDD who did not receive ECT ($N=3393$).

ECT was performed using a Thymatron IV ECT device. All patients received bifrontal (BF) ECT, with stimulus dose administration at least 200% above the seizure threshold “based on age-based method”. ECT was administered three times a week with an average of 6 ± 1 ECT sessions. Anesthesia consisted of pretreatment with glycopyrrolate, followed by induction with an anesthetic agent (methohexital, 0.75 mg/kg; propofol 1 mg/kg in 5 cases) followed by succinylcholine (0.75 mg/kg) for muscle relaxation.

Improvement of PTSD and MDD symptoms, as assessed by clinical global impression scale (CGI) was better for ECT (90%), compared to antidepressant medications (50%) ($P = .001$). Over a mean follow-up period of 8 years, they found that the ECT-treated group had statistically significant risk reductions of 64% for suicide, 65% for all-cause mortality, and 46% risk reduction for cardiovascular mortality relative to the group that did not receive ECT. Symptomatic improvement was also greater in the ECT-treated patients.

TABLE 1

Electroconvulsive therapy studies in posttraumatic stress disorder

Author(s)/years of the study period	N	Design	Sample	EP and (XST)	PW	Definition of response and remission
Margoob et al (2010) (January 1, 2005, to December 31, 2005) ²⁸	20	Open-label, prospective study	All “consenting” adults with PTSD (DSM-IV), outpatients All eligible outpatients undergoing ECT participated. Age range: 21 to 61 years; mean 38.0 (SD 12.1); 50% males; Duration of PTSD ranged from 18 to 95 months, mean 35.9 (SD 18.7) months	Fixed course of 6 BL, twice-weekly outpatient	1.5 ms	30% reduction in CAPS
Watts (2007) (January 1998 to December 2002) ²⁹	26	Retrospective chart review	Veterans with PTSD and comorbid MDD (DSM-IV)	All started RUL (2.5); 10 switched to BL	–	Depression, response 50% reduction on MADRS, remission ≥60% reduction and a final MADRS + ≤10 score. PTSD response ≥20% reduction on PCL
Ahmadi et al (2016) (2004 to 2013) ³²	92 received ECT, 3393 did not receive ECT	Retrospective, nested, with matched case-control	Patients with PTSD and comorbid MDD who had (n = 92) and had not (n = 3393) received ECT. Mean study period was 8 years	Bifrontal ECT (at least 200% XST)	–	–

BL: bilateral ECT; CAPS: Clinician-Administered PTSD scale, CGI-S: Clinical Global Impression-Severity; ECT: electroconvulsive therapy; EP: electrode placement; MADRS: Montgomery-Åsberg Depression Rating Scale; MDD: major depressive disorder; ms: milliseconds; PCL: PTSD Checklist; PTSD: posttraumatic stress disorder; PW: pulse width; RUL: right unilateral ECT; SD: standard deviation; XST: times above seizure threshold.

Primary outcome measure(s)	Changes in primary outcome measure(s)
CAPS Secondary: MADRS, CGI-S	A fixed course of 6 bilateral ECT sessions resulted in a CAPS improvement of 34%, and MADRS improvement of 51%. Most improvement in both CAPS and MADRS occurred by the third ECT session. It was noted that the response rate was 70% in the intention-to-treat analysis and 82% in completers; no patients remitted. Treatment gains were maintained at a 4 to 6 month follow-up. However, this was assessed clinically and not by rating scales
PCL, MADRS	Although the symptoms of MDD were reduced significantly, symptoms of PTSD showed some improvement. "The subjects showed a reduction in PTSD, as measured using the PCL, from 71.08 (SD, 4.72) before ECT to 55.62 (SD, 8.84) after ECT ($P > .001$). Thirty-five percent of subjects showed a 20% reduction in symptoms, meeting the established criterion for PTSD response". The response rate for MDD was 50%, and the remission rate was 42%
Symptomatic improvement using CGI-S and change scales at baseline and 3 month follow up The long-term end points were occurrence of cardiovascular, all-cause mortality, and suicide	Symptomatic improvement was greater in the ECT-treated patients. Moreover, ECT-treated group had a risk reduction of 64% for suicide, 65% reduction for all-cause mortality, and 46% reduction for cardiovascular mortality compared with those who did not received ECT

Case reports

Our search also produced 5 case reports on the use of ECT in individuals with PTSD. The first case describes a 35-year-old woman who had severe combat-related PTSD and depression. The PTSD symptoms were resistant to standard therapies of both medication and psychotherapy (Helsley, Sheikh, Kim, & Park, 1999). The patient underwent an acute course of 6 unilateral ECT sessions (3 times per week) after discontinuation of all psychotropic medications. PTSD symptoms responded "substantially" to the acute ECT course.

Another case depicts a 38-year-old woman who had medication-refractory depression and PTSD. The patient also had complete remission of depression and PTSD symptoms after 8 bilateral ECT sessions (Hanretta & Malek-Ahmadi, 2006). In both of the above cases, no rating

scales were used, but rather diagnoses and symptoms remission were based on clinical assessment (Table 2).

A third recently published case report utilized conversation-based reactivation of traumatic memory contents in PTSD and subsequent ECT-mediated impairment of reconsolidation (Gahr, Schonfeldt-Lecuona, Spitzer, & Graf, 2014). The patient also was followed for 12 weeks after the last ECT session. The patient was assessed with rating scales for PTSD (Clinician-Administered PTSD scale, Part 2 [CAPS-2])(Blake et al., 1995) and depression (Beck Depression Inventory II [BDI])(Beck, Steer, & Brown, 1996), MADRS, and Hamilton Rating Scale for Depression (Hamilton, 1960).

It might be argued that the efficacy of ECT in PTSD is due to impaired memory and thus impaired reactivation of traumatic memories. Memory contents are thought to be destabilized during retrieval of such memories, lending them sensitive to modifications (Schwabe, Nader, Wolf, Beaudry, & Pruessner, 2012). This was the premise for this case, assuming that ECT might be “most effective immediately after an active (conversation-based) confrontation” with traumatic memories.

The patient received 8 sessions of right unilateral ECT (3 sessions per week). The ECT parameters and device were not specified. The authors also reported that they “did not observe any significant side effects related to ECT”. Sertraline, 200 mg/d, and trimipramine, 75 mg/d were co-administered during the ECT procedure (and after the ECT course). The patient had PTSD that was treatment resistant for 7 years to both psychotherapy and pharmacotherapy. After ECT, the patient experienced improvement in PTSD symptoms. Depressive symptoms also improved. The authors explain the improvement is due to possibly “successful ECT-mediated extinction” of traumatic memory.

The fourth and fifth cases were published by Ozdemir et al. (Ozdemir et al., 2013). The fourth case presents a 40-year-old male who developed PTSD and psychotic disorder not otherwise specified (NOS) after combat per DSM-IV-TR. He suffered a range of symptoms including recurring nightmares, hyper-arousal, and insomnia as well as auditory and visual hallucinations, persecutory delusions, and disorganized and violent behaviors. After hospitalization, the patient was started on risperidone 4 mg/d and venlafaxine 225 mg/d. There was no improvement after 6 weeks of pharmacotherapy, thus he was offered a course of 10 sessions of bilateral ECT, 3 sessions per week, along with the same doses of the medications, using the Thymatron device (System IV; Somatics, Lake Bluff, Ill), with a stimulus titration schedule beginning at 25% (charge, 126 mC; frequency, 30 Hz; duration, 2.33 s; current, 0.9 A; and pulse width, 1.0 ms) until seizure threshold of 60% was attained. Each stimulus resulted in seizure duration ranging from 20 to 45s. (It was not specified if subsequent treatments were subsequent treatments at threshold, or at a higher level.) Psychotic and PTSD symptoms improved, and the authors reported that after the 10th session of ECT, both PTSD and psychotic symptoms resolved. Brief Psychiatric Rating Scale (BPRS) score improved by 33.6% (baseline 44), and the CAPS improved by 37.3% (baseline 67).

The fifth case also presents a 42-year-old man diagnosed with PTSD and psychotic disorder NOS after combat-related trauma, according to DSM-IV criteria. The patient presented with re-experiencing traumatic events, auditory and visual hallucinations, persecutory delusions,

social isolation, and hearing sounds of dead soldiers. He was admitted for hospitalization and also was started on risperidone, 4 mg/d, and venlafaxine, 225 mg/d. There was “insignificant improvement” with medications for 6 weeks. Subsequently, the patient underwent ECT (8 sessions, electrode placement not mentioned) along with the medication. Both PTSD and psychotic symptoms improved after the 8 ECT sessions. Baseline BPRS score of 42 improved by 35.2%, and baseline CAPS score of 66 improved by 37.2%. Two weeks after the ECT course, the dose of risperidone was reduced to 1 mg/d. The symptoms continued to improve with a decrease in BPRS by 65.1% and a decrease in CAPS by 44.5% from baseline.

TABLE 2
Electroconvulsive therapy case reports in posttraumatic stress disorder

Study	Age, sex, N	Diagnosis	ECT course	ECT device/parameters	Psychotropics during the ECT course
Özdemir et al (2013) ²⁷	40-year-old male	Combat-related PTSD and comorbid psychosis NOS	10 BL ECT sessions	Thymatron device IV. Titration schedule beginning at 25% with charge, 126 mC; frequency, 30 Hz; duration, 2.33 seconds; current, 0.9 A; and pulse width, 1.0 ms was increased until seizure threshold of 60% was attained. Each stimulus resulted in seizure duration from 20 to 45 s	Risperidone, 4 mg/d, and venlafaxine, 225 mg/d
	42-year-old male	Combat-related PTSD and comorbid psychosis NOS	8 ECT sessions; electrode placement not mentioned	Not mentioned	Risperidone, 4 mg/d, and venlafaxine, 225 mg/d
Heisley et al (1999) ³³	35-year-old woman	Severe combat-related treatment-resistant PTSD and depression	Index course of 6 unilateral ECT, 3 per week	---	Discontinuation of all psychotropic medications
Hanretta and Malek-Ahmadi (2006) ³⁴	38-year-old woman	Medication-refractory depression and PTSD	8 BL ECT sessions	Thymatron DG. Energy dial was set at 40% (frequency, 50 Hz; duration, 2.24 seconds; number of pulses, 224; charge, 201.6 mC) Seizure duration range, 26 to 44 seconds	Combination of duloxetine, 60 mg/d, and olanzapine, 15 mg/d
Gahr et al (2014) ³⁵	49-year-old man	Psychotherapy and pharmacotherapy-refractory PTSD for 7 years (PTSD assessed with CAPS-2) ³⁶	Conversation-based reactivation of traumatic memory contents in PTSD, followed by 8 RUL ECT sessions (3 per week)	ECT parameters and device were not specified	Combination of sertraline, 200, mg/d, and trimipramine, 75 mg/d

BL: bilateral; BPRS: Brief Psychiatric Rating Scale; CAPS-2: Clinician-Administered PTSD scale, Part 2; ECT: electroconvulsive therapy; ms: milliseconds; NOS: not otherwise specified; PTSD: posttraumatic stress disorder; RUL: right unilateral.

DISCUSSION

This review found limited publication on the treatment of PTSD, mainly treatment-resistant PTSD, using ECT. The available preliminary data suggest modest improvement in the severity of PTSD symptoms, especially in patients with concurrent PTSD and MDD. The improvement is not correlated merely with improvement in depressive symptoms (as might be thought intuitively).

A sizable portion of patients with PTSD is treatment-resistant (Brady et al., 2000; Davidson, Rothbaum, van der Kolk, Sikes, & Farfel, 2001; Youssef et al., 2012). In non-treatment-resistant patients, response rates to approved pharmacotherapy are 50% to 60% at best (Brady et al., 2000; Davidson, Tharwani, & Connor, 2002; Marshall, Beebe, Oldham, & Zaninelli, 2001; Tucker et al., 2001). In the only clinical trial and the most rigorously available in the literature so far, by Margoob et al. (Margoob et al., 2010), the intention-to-treat response rate to ECT for PTSD in this pharmacotherapy- and psychotherapy-resistant sample was 70%. If that holds in a larger controlled trial, it will offer substantial promise for treatment-resistant PTSD patients. Also, the participants had improvements after 6 ECT sessions. It is also possible that treatment response would be maintained or improved after a longer acute course analogous to the standard for MDD. Finally the techniques of memory reactivation before ECT procedure may be another important direction of research (Gahr et al., 2014).

There are several limitations regarding the conclusions that can be drawn from the available literature. First, this systematic review is limited by the small number of published reports and the small number of participants in most studies. It is also limited by the design of retrospective studies and one open-label prospective studies, and by the lack of randomized clinical trials. Second, it is hard to rule out the potential placebo response of ECT on PTSD symptoms in such studies with a small sample size and non-controlled studies, as well as the potential effect of hospitalization while receiving psychosocial treatment. Third, the possibility of publication bias (in which studies with negative outcomes are not published) could not be ruled out, especially in the absence of rigorously done randomized controlled trials in the literature.

As a result of these limitations, it is unclear whether ECT is effective for PTSD. As there is not enough evidence to support its efficacy so far, it is hard to ascertain if PTSD symptoms have improved only because of improvement in depression or if there is a true anti-PTSD effect. However, there is no evidence of it causing harm either. It is possible that a subset of patients with PTSD will respond to ECT. Moreover, accumulating evidence supports the notion that continuing antidepressants during ECT improves MDD outcomes (Youssef & McCall, 2014). Similarly, it is probable that discontinuation of antidepressants in the 2 studies of the use of ECT in PTSD available in the literature (as well as in a case report) might have interfered with ECT outcomes for these patients. More rigorous and well-powered controlled clinical trials are needed, however, for more conclusive and more definitive results.

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Chapter 12 Feasibility, Safety, and Preliminary Efficacy of Low Amplitude Seizure Therapy (LAP-ST): A Proof of Concept Clinical Trial in Man

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Abstract

Background: Current pulse amplitude used in clinical ECT may be higher than needed. Reducing pulse amplitude may improve focality of the electric field and thus cognitive adverse effects. Here we examine the feasibility, safety, and whether Low Pulse Amplitude Seizure Therapy (LAP-ST, 0.5-0.6A) minimizes cognitive adverse effects while retaining efficacy.

Methods: Patients with treatment-resistant primary mood (depressive episodes) or psychotic disorders who were clinically indicated to undergo ECT were offered to be enrolled in an open-label study. The study consisted of a full acute course of LAP-ST under standard anesthesia and muscle relaxation. The primary outcome was feasibility of seizure induction. Clinical outcome measures were: time to reorientation (TRO), Mini Mental State Examination, Montgomery Aberg Depression Scale, and Brief Psychiatric Rating Scale, and Clinical Global Impression Scale.

Results: Twenty-two patients consented to enrollment in the study. LAP-ST was feasible, and all patients had seizures in the first session. Participants had a quick orientation with median TRO of 4.5 minutes. Treatment was efficacious for both depressive and psychotic symptoms.

Limitations: Relatively small sample size, non-blinded, and no randomization was performed in this initial proof of concept study.

Conclusions: This first human preliminary data of a full course of focal LAP-ST demonstrates that seizure induction is feasible. These results, although preliminary, suggest that the LAP-ST compared to the standard ECT techniques may result in less cognitive side effects, but comparable efficacy. Larger studies are needed to replicate these findings.

Keywords

Electroconvulsive therapy; seizure therapy; low amplitude seizure therapy; ECT; seizure threshold, current titration; focal ECT; treatment-resistant depression; mood disorders; psychotic disorders.

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INTRODUCTION

Mood and psychotic disorders have a huge negative impact on morbidity and mortality from mental and physical illness, with a substantial burden on the health of individuals, families, and the society (Murray & Lopez, 1997; Szkulitecka-Debek et al., 2016). Depression is predicted to be the second highest cause of disability after ischemic heart diseases by year 2020 (Murray & Lopez, 1997). Treatment-resistant depression (TRD) was defined by the World Psychiatric Organization and the Massachusetts General Hospital Depression Program as failure to respond to an adequate trial of medication (Ayd, 1983; Fava & Davidson, 1996). However, about a third of patients do not achieve remission despite 3 lines of adequate treatment trials as shown by the STAR*D clinical trial (Rush et al., 2006; Subedi, Aich, & Sharma, 2016; Szkulitecka-Debek et al., 2016). ECT is used for treatment-resistant schizophrenia usually in combination with antipsychotics (Tharyan & Adams, 2005).

Electroconvulsive therapy (ECT) has been the most potent (UK ECT Review Group, 2003) and most rapid acting (Husain et al., 2004) form of intervention especially for the treatment-resistant mood and psychotic disorders in combination with medications (Griffiths, Zarate, & Rasimas, 2014; Kellner, Husain, Knapp, McCall, Petrides, Rudorfer, Young, Sampson, McClintock, Mueller, Prudic, Greenberg, Weiner, Bailine, Rosenquist, Raza, Kaliora, Latoussakis, Tobias, Briggs, Liebman, Geduldig, Teklehaimanot, Lisanby, et al., 2016; Levy-Rueff, Jurgens, Loo, Olie, & Amado, 2008; Medda et al., 2015; Minnai, Salis, Manchia, Pinna, & Tondo, 2016; Palma et al., 2016; Prudic & Sackeim, 1999; Subedi et al., 2016; Tharyan & Adams, 2005; Youssef & McCall, 2014). ECT can also help prevent relapse in treatment-resistant depression with continuation ECT and medications (Kellner, Husain, Knapp, McCall, Petrides, Rudorfer, Young, Sampson, McClintock, Mueller, Prudic, Greenberg, Weiner, Bailine, Rosenquist, Raza, Kaliora, Latoussakis, Tobias, Briggs, Liebman, Geduldig, Teklehaimanot, Dooley, et al., 2016; Youssef & McCall, 2014).

Nonetheless, cognitive and memory-related adverse effects of ECT are still a major concern. Historically, modifications in ECT parameters in the form of ultra-brief pulse instead of brief pulse (Cronholm & Ottosson, 1963a, 1963b) and the use of rectangular instead of sine wave (L. R. Squire & Zouzonis, 1986; Weiner, 1980), as well as right unilateral (RUL) electrode placement instead of bilateral (BL) (L. R. Squire, 1977; S. R. Squire & Slater, 1978) have lowered cognitive and memory adverse effects (Sackeim et al., 1986; Sackeim et al., 1993; Sackeim, Prudic, et al., 2000; Sackeim et al., 2008). However, despite these advances, memory and cognitive side effects are still experienced by a significant proportion of patients, constitute a barrier to treatment, and may, in part, contribute to the stigma associated with ECT (Matthews, Rosenquist, & McCall, 2016; Sienaert, 2016). Thus, ECT is still underutilized as an effective life-saving intervention (Prudic & Sackeim, 1999), despite the fact that ECT, when indicated, has a great impact on the quality of life and functionality of patients (McCall et al., 2013; McCall et al., 2011).

The current amplitude of the electric pulse is the primary factor that drives the electric field deeper in the brain structures (Peterchev, Krystal, Rosa, & Lisanby, 2015; Peterchev, Rosa, Deng, Prudic, & Lisanby, 2010). Thus, Low Amplitude ECT, or more precisely Seizure Therapy (LAP-

ST), should result in minimal spread of the electric field in the deeper hippocampal region and temporal lobe. (Peterchev et al., 2015; Peterchev et al., 2010). Avoidance or minimization of hippocampal and temporal lobe stimulation using LAP-ST is hypothesized to significantly reduce memory and cognitive adverse effects of ECT without affecting the more superficial stimulation of the Dorsolateral Prefrontal Cortex (DLPFC) and other cortical regions needed for the antidepressant effect (Noda et al., 2015).

Computational models of electric field induced in the brain using conventional high current amplitude is much higher than the neural activation threshold. This leads to deeper spread of the electric field in the brain. On the other hand, reducing pulse amplitude improves focality and decreases depth of penetration, thus potentially minimizing memory and cognitive adverse effects (Lee, Lisanby, Laine, & Peterchev, 2012).

Low amplitude has been utilized in older ECT paradigms, (Friedman, 1942; Liberson, 1948) without anesthesia using older devices that have different specifications and parameters, such as being voltage constant (which leaves the current pulse amplitude to vary from patient to patient and session to session depending on the impedance). Modern ECT devices are current-constant rather than voltage-constant. This allows for true fixed current amplitude. Thus, constantly minimizing the propagation of electric fields to the deeper memory areas of the brain that is associated with the cognitive adverse effects of standard ECT. Currently, in standard clinical practice, a high pulse amplitude of 800 to 900mA is used (Lee, Lisanby, Laine, & Peterchev, 2016). Also, in these older studies, anesthesia was not used, which would affect the seizure threshold (ST) estimates (Avramov, Husain, & White, 1995).

In modern era, seizure titration during the first session using low amplitude was successfully reported in a letter by Rosa et al 2011 on 5 cases (Rosa, Abdo, Lisanby, & Peterchev, 2011). These case reports involved one titration session, but no suprathreshold therapeutic dose sessions using low amplitude ECT.

Nahas et al performed a study to primarily examine investigational Focal Electrically Administered Seizure Therapy (FEAST) electrode placement and investigational unidirectional pulse (Nahas et al., 2013). In this study, low pulse amplitude were used to decrease an unusually high impedance at the smaller FEAST electrode. However, the primary aim of the study was to examine a novel electrode placement (along with a unidirectional current).

Although ECT has been in clinical practice for decades, so far, no human studies have primarily examined the feasibility or the effect of lower pulse amplitude of a full course on memory and cognition. Our hypothesis is that LAP-ST will reduce the cognitive burden of ECT. We also hypothesized that LAP-ST will be efficacious in both depressive and psychotic episodes. The aims of this study were to first examine the feasibility (primary outcome) of a full course of LAP-ST using low amplitude in humans and whether LAP-ST is associated with less cognitive adverse effects using Time to Orientation (as the main clinical outcome). We also explored the efficacy of LAP-ST.

METHODS

Participants.

After IRB approval, patients were offered to be enrolled in the study if they were clinically indicated to undergo ECT, had capacity to consent for the study, and provided written informed consent. This study included patients with either primary mood or psychotic disorders who failed to respond to clinically adequate trials of psychopharmacological interventions in term of dosage and duration (with or without psychotherapy) as clinically indicated and were referred for ECT in Behman hospital in Cairo, Egypt. All patients were inpatients. Behman Hospital is a private psychiatric hospital accredited by the Royal College of Psychiatrists in England. Patients were enrolled between April 2013 to June 2015 after explanation of potential risks and any possible generalizable benefits of the study. The open-label study design involved a clinical course of LAP-ST typically given 3 times a week under standard anesthesia (sodium thiopental) and muscle relaxation (succinylcholine). There were no set minimum or maximum numbers of sessions.

Eligibility.

Inclusion criteria for enrollment were as follows: 1) ECT clinically indicated, 2) Males or females 22 to 80 years of age, 3) Use of effective method of birth control for women of child-bearing capacity, 4) Patient is medically stable (i.e. no serious acute medical condition, generally assessed within the week before the ECT administration and on the morning of the ECT), 5) Capacity of patient to fully participate in the informed consent process as evaluated by their clinician.

Exclusion criteria included the following: 1) Current unstable or serious medical condition, or any co-morbid medical condition that substantially increases the risks of ECT, 2) History of conditions that may make ECT or anesthesia unsafe, 3) Female patients who are currently pregnant or plan to be pregnant during the study.

Patients were allowed to continue on their medications as clinically appropriate but on a fixed dose during the study.

Assessments.

Participants were diagnosed clinically according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM IV) criteria. Response and remission was determined clinically. Participants were also evaluated using Montgomery-Åsberg Depression Rating Scale (MADRS) (to measure change in depressive symptoms), Mini-Mental State Examination (for global cognitive function assessment) using standard Arabic translation (Al-Rajeh, Ogunniyi, Awada, Daif, & Zaidan, 1999; Folstein, Folstein, & McHugh, 1975) at baseline and endpoint. The 14-question Time to Reorientation test (TRO, the main cognitive measure) assessed the time to reorientation after ECT by asking 14 questions for person specific information (e.g., name, age), time information (e.g., date) and place information (e.g., location). The items are asked at 3, 5,

10, 15, and 20 minutes following the end of seizure expression as documented on the EEG recording. The TRO score is the time it takes the patient to be able to answer 14/14 correctly. This test was modeled after and used by the PRIDE study workgroup (Kellner, Husain, Knapp, McCall, Petrides, Rudorfer, Young, Sampson, McClintock, Mueller, Prudic, Greenberg, Weiner, Bailine, Rosenquist, Raza, Kaliora, Latoussakis, Tobias, Briggs, Liebman, Geduldig, Teklehaimanot, Lisanby, et al., 2016). In addition to baseline and endpoint assessments, MADRS and MMSE were also done before each session, and TRO after each session. Patients diagnosed with schizophrenia or schizoaffective were also evaluated with Brief Psychiatric Rating Scale to measure change in psychotic symptoms (BPRS). Clinical Global Impression severity score (CGI-S) was used to assess overall change in clinical severity of symptoms. These scales were administered by an experienced staff psychiatrist (E.S). General side effects were assessed clinically by asking the patient if they have any side effects, or if patients simultaneously reported side effects that may be related to the ECT administration.

Procedure.

Patients received the standard clinical pre-ECT medical and laboratory workup as indicated by the treating clinicians for safe delivery of ECT and anesthetic procedures. Medication and anesthesia adjustments were used according to the discretion of the treating psychiatrist and ECT trained anesthesiologist as deemed clinically necessary according to the standard of care. A bite-block was inserted prior to seizure elicitation to protect the teeth. All other standard safety procedures for ECT were done as per the American Psychiatric Association ECT Guideline (American Psychiatric Association) (*The Practice of Electroconvulsive Therapy: Recommendations for Treatment, Training, and Privileging: A Task Force Report of the American Psychiatric Association. (2001)*). LAP-ST treatments during the acute course of this study were given three times per week (Kellner, Husain, Knapp, McCall, Petrides, Rudorfer, Young, Sampson, McClintock, Mueller, Prudic, Greenberg, Weiner, Bailine, Rosenquist, Raza, Kaliora, Latoussakis, Tobias, Briggs, Liebman, Geduldig, Teklehaimanot, Lisanby, et al., 2016).

Low Amplitude Seizure Therapy (LAP-ST).

LAP-ST was performed using Mecta Spectrum 5000Q device and Right Unilateral (RUL) electrode placement. All seizure titrations were planned during the first session.

For seizure titration (ST) in the first session: Stimulus parameters were fixed at pulse width of 0.3ms and duration of 8s. The seizure threshold was titrated using the current first from 500 to a maximum of 600mA that was allowed in the study. Then, the frequency of the stimulus was titrated up if seizures were not induced; starting with frequency of 20Hz. Subsequent sessions change were 6 times that of the seizure threshold (that was determined in the first session). Tables 2 and 3 present further details of electric parameters, findings, duration of motor and EEG seizures, and anesthesia and muscle relaxation dosage.

Anesthesia.

Anesthesia was administered by an anesthesiologist with specific training and experience in ECT, as per standard anesthesia used for clinical ECT and the Hospital Policy and Procedure. Briefly, blood pressure, pulse, ECG, and pulse oximetry were monitored prior to anesthetic induction and continuously during the procedure. Standardized anesthesia procedures include induction with sodium thiopental 250 mg and muscle relaxation with succinylcholine (~0.75 mg/kg) or as determined clinically appropriate by the anesthesiologist. Patients were ventilated with 100% oxygen from anesthetic administration until the return of spontaneous respirations. Seizure expression was monitored by left fronto-mastoid EEG, and inspection of the cuffed foot to record motor manifestations. Both the duration of the EEG seizure and motor seizure duration were recorded in seconds.

Electroencephalography (EEG) Acquisition and ictal monitoring.

EEG seizure duration was monitored using two EEG channels (fronto-mastoid electrodes). The duration of the seizure in the brain was determined by the EEG recording (**American Psychiatric Association**) (*The Practice of Electroconvulsive Therapy: Recommendations for Treatment, Training, and Privileging: A Task Force Report of the American Psychiatric Association. (2001)*). Motor manifestations of seizures were monitored using the cuff technique. The cuff technique entails the placement of a blood pressure cuff on a limb. The cuff is then inflated above the systolic blood pressure immediately prior to the infusion of succinylcholine. Motor manifestations were observed and also monitored by Optical Motion Sensor (OMS) in combination with inspection of other cuffed foot and part of the body for motor seizures.

Data analyses.

The data was visualized and assessed for normality (i.e. normal distribution). Based on the normality of the data, or lack of normality, it was decided *a priori* to either use parametric or non-parametric tests, respectively. Due to the data not being normally distributed, it was reported using median and quartiles and compared by Wilcoxon Signed Ranks Test for paired data of the pre-post scores. TRO was reported with median and mean (to compare with historical controls). Wilcoxon Signed Ranks Tests were two tailed, and alpha was set *a priori* at $P = 0.05$. SPSS version 23, (Inc., Chicago, IL, USA) was used for the statistical analyses. LOCF analysis was used in this pilot study.

RESULTS

Demographic and clinical characteristics.

Data from the total sample of 22 subjects are summarized in Table 1. Fifty percent (11) of participants from the total sample had a primary mood disorder diagnosis, and the other 50% had a primary psychotic disorder diagnosis as described in Table 1. All subjects, except for one,

were taking psychotropic medication. The doses of the psychotropic medications were kept constant during the LAP-ST course.

Treatment characteristics, parameters, and seizure duration.

The dose of electrical stimulation and the electric parameters of the first titration session are described in Table 2, and those of subsequent suprathreshold sessions (sessions at 6 times seizure threshold) are described in Table 3. Patients were treated as determined clinically to respond, remit, decide to withdraw from the study, or were clinically determined as non-responders by the ECT psychiatrist.

No patients had comorbid substance use disorder. This was determined by clinical history as well as negative drug screens. None of the patients were on prescribed benzodiazepines as per the hospital policy of not prescribing benzodiazepines during an ECT course.

Twenty-two patients underwent acute course of LAP-ST. All patients had seizure in the first session. No patients required more than 2 stimuli at the first session for seizure induction. The median number of sessions for the total sample was 6.

Feasibility and safety.

The study showed feasibility of LAP-ST during a full course in humans. Threshold and suprathreshold seizures at 6 times seizure threshold were successfully induced in all patients.

One patient subjectively mentioned that for a few minutes after reorientation he had trouble focusing, however it was difficult to discern whether that was the effect of anesthesia and/or ECT. No participants subjectively complained of memory side effects during or after the LAP-ST course. Subjective memory problems were simply assessed by asking patients if they think they have any memory problems during or after ECT and as a result of it.

There were 3 patients who had headaches in the initial session which remitted after taking prophylactic ibuprofen the night before ECT sessions. Beyond that, there were no other reported or observed adverse effects related to the study.

Cognitive assessment.

The suprathreshold sessions for all participants during the LAP-ST course (excluding the first titration session) had a median time to full orientation (TRO) of 4.5 min (IQR = 3.2-10, N=20), and mean of 6.6 minutes.

Median MMSE at baseline was 29.5 (IQR=27.8-30) and post LAP-ST was 30 (IQR=28-30), indicating no statistically significant difference ($p=0.6$, $z=0.6$, $N=18$ paired data, Wilcoxon Signed Ranks Test).

Clinical efficacy.

In patients who had a LAP-ST course of more than one suprathreshold treatment session (i.e. subsequent sessions at 6-times seizure threshold), the median Clinical Global Impression-Severity scales (CGI-S) improved from baseline of 6 down by 4 points (IQR =2.5-4.5) for patients in a depressive episode. For patients with schizophrenia, CGI-S improved from baseline of 5 down by 2.5 points (IQR = 1.25-3.0). CGI-S score for the total sample improved from baseline median of 5.5 (IQR = 4-6) to endpoint of 3. However, this non-primary outcome (CGI-S) has some missing data and pre-post data for CGI were available for only 14 participants. The improvement in CGI-S was statistically significant ($p=0.002$, $z=-3.06$).

On the MADRS, depression significantly improved ($p=0.018$, $z=-2.4$, Wilcoxon Signed Ranks Test) in patients with unipolar or bipolar depression from baseline (median=34, IQR=12-46.5) to LAP-ST endpoint (median=10, IQR=6-36.5).

Psychotic symptoms (on BPRS) also significantly improved (Wilcoxon Signed Ranks Test $p=0.005$, $z=-2.8$). The median improvement was 27 (IQR=21.5-32.3) in patients with schizophrenia or schizoaffective disorders from 73.5 (IQR=62-86.3) before LAP-ST to 47 (IQR=36-52.8) after the LAP-ST.

DISCUSSION

This proof of concept study demonstrates that seizure induction using standard RUL electrode placement with focal LAP-ST course is both feasible and safe (with no significant side effects) in humans. This is considerable given the use of lower pulse amplitude along with thiopental, which probably leads to a higher seizure threshold compared to methohexital (Fond et al., 2016; Lihua, Su, Ke, & Ziemann-Gimmel, 2014) (the most common anesthetic used in the US for ECT). In addition, as hypothesized LAP-ST seems to have reduced cognitive adverse effects (as measured by the TRO and MMSE). These data are promising and represent the first LAP-ST course in humans in modern times.

Similarly, in this preliminary study, efficacy was robust for both depressive and psychotic symptoms and seems comparable to studies of standard ECT. However, it should be noted that this proof of concept study warrants replication.

Acute cognitive side effects with LAP-ST versus standard ECT.

Previous studies found that longer TRO is prospectively correlated with more long-term memory side effects with ECT (Martin, Galvez, & Loo, 2015; Sackeim, Luber, et al., 2000; Sobin et al., 1995). TRO reported in published studies using standard ECT, with both RUL and bilateral ECT, were much longer (Kellner, Husain, Knapp, McCall, Petrides, Rudorfer, Young, Sampson, McClintock, Mueller, Prudic, Greenberg, Weiner, Bailine, Rosenquist, Raza, Kaliora, Latoussakis, Tobias, Briggs, Liebman, Geduldig, Teklehaimanot, Lisanby, et al., 2016; Kellner et al., 2010; Loo et al., 2014; Sackeim, Luber, et al., 2000; Sackeim et al., 2008) than the current study, predicting more memory adverse effects with standard ECT than with LAP-ST.

For instance, in a study by Sackeim and colleagues, 59 patients were randomized to either standard RUL or BL ECT administered at either “low dose” (just above ST) or “high dose” (150% above ST). In this study, TRO was defined as the time after the end of seizure when patients could correctly answer 4 of 5 questions. Orientation questions were “presented continuously until positive response.” The mean TRO ranged from 10.8 min (SD=8.3) for RUL low dose, 21.2 min (SD=11.8) for RUL high dose, 39.4 min (SD=20.9) for BL low dose and 42.7 min (SD=16.0) for BL high dose ECT (Sackeim, Luber, et al., 2000). It should be noted that, generally speaking, low dose ECT means low total charge which is achieved by lowering the other electric parameter (such as the frequency or the duration of the stimulus) but not the current amplitude, while the current amplitude stays fixed at 800 or 900mA, depending on the device used in each study.

In another study by Kellner and colleagues (Kellner et al., 2010), reorientation score at 20 min after ECT was assessed using a ten-question instrument. Participants were randomized to receive bifrontal or BL ECT at 1.5 times above ST, or RUL ECT at 6 times above the ST. Averaged across all ECT sessions, except session one, it was reported that “the three electrode placements were not statistically different.” The orientation time was assessed at 20 min. The average questions answered correctly out of a total 10 questions were only 4.8 (SD = 2.5) questions for bifrontal ECT, 5.7 (SD= 2.5) for RUL, and 5.5 (SD = 2.8) for BL (bitemporal). At 20 min, none of the electrode placement out of the 3 using high current amplitude was close to the full score of 10/10 correct questions.

Time to reorientation in other more recent published studies using ultra-brief pulse RUL ranged from 10–15 min, and for brief pulse RUL TRO ranged from 21–22 min (Kellner, Husain, Knapp, McCall, Petrides, Rudorfer, Young, Sampson, McClintock, Mueller, Prudic, Greenberg, Weiner, Bailine, Rosenquist, Raza, Kaliora, Latoussakis, Tobias, Briggs, Liebman, Geduldig, Teklehaimanot, Lisanby, et al., 2016; Loo et al., 2014; Sackeim et al., 2008). In a recent large clinical trial, the Prolonged Remission In Depressed Elderly (PRIDE), ultra-brief RUL ECT protocol was used for ECT in all participants reporting “relatively rapid reorientation, with close to half of patients being reoriented by 10 minutes.” (PRIDE) (Kellner, Husain, Knapp, McCall, Petrides, Rudorfer, Young, Sampson, McClintock, Mueller, Prudic, Greenberg, Weiner, Bailine, Rosenquist, Raza, Kaliora, Latoussakis, Tobias, Briggs, Liebman, Geduldig, Teklehaimanot, Lisanby, et al., 2016). Thus, it seems obvious that lower amplitude during a full course, as performed in this LAP-

ST, provided even further benefit beyond standard ultra-brief RUL ECT that used high amplitude ECT.

Of note, the present study used more orientation questions (14 questions) and more rigorous cutoff to full orientation (14 out of 14 questions answered correctly) than many of the published studies, making a shorter TRO harder to achieve. Those who answered all 14 questions correctly did that in a mean TRO of 6.6 min and a median of 4.5 in the present study. Despite that, the present LAP-ST study had no participants who had mean TRO as long as 20 min.

The mean MMSE scores at the end of the LAP-ST course showed no impairment, as an overall assessment of cognition throughout the LAP-ST course. In ECT using standard amplitude, MMSE scores typically show decline (Kellner et al., 2010; Sackeim, Luber, et al., 2000). For instance, in the one study, the mean MMSE dropped by 1.6 points from baseline to post ECT (Kellner et al., 2010). However, it should be noted that MMSE is not a sensitive measure of cognitive change and memory side effects in ECT literature, but that the decline in a short period (during one course) using standard ECT could also have some clinical importance. This could indicate even more significant clinical impairment in cognition over more frequent number of sessions or with multiple ECT courses (as in case of some treatment-resistant patients). Nonetheless, TRO is a more sensitive measure and predictive of memory side effects as explained earlier.

Weaknesses of the present study, however, include the use of only TRO and MMSE for assessment of cognition without additional cognitive tests. This was done due to the preliminary nature and limited budget of the study. As mentioned, however, TRO has been reliably shown in the ECT literature to correlate with memory problems prospectively (Lisanby, Luber, Schlaepfer, & Sackeim, 2003; Sackeim, Luber, et al., 2000; Sackeim et al., 2008; Sobin et al., 1995). More extensive neurocognitive testing is warranted in larger studies. Secondly, this study had a relatively small sample size and was open-label and non-randomized. Further investigations using randomized clinical trial design with a larger sample size are warranted to support this first study in humans.

Nonetheless, data from this proof of concept study is very promising for the potential future utility of LAP-ST for treatment-resistant mood and psychotic disorders. A focal LAP-ST course seems to be efficacious with less memory side effects (as predicted by TRO) when compared to standard ECT. This line of research could substantially move the field forward in terms of reducing cognitive burden of ECT and offers groundwork for definitive studies for more focal seizure therapy.

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Contributors: *Dr. Nagy Youssef conceptualized and designed the study, wrote the protocol including the electric parameters and titration for LAP-ST, IRB submission, advised and consulted on study implementation, analyzed the data, and wrote the manuscript. Dr. Emad Sidhom helped with the addressing IRB questions and implemented all the study, administered the LAP-ST as the ECT psychiatrist, collected and entered the data and helped in editing the manuscript. All authors have approved the final article.*

Table 1. Subject demographic and clinical characteristics (N=22).

Age, mean (SD), years	41(15.2)
Median	38
Sex, Female, N (%)	16 (73%)
Marital Status, N (%)*	
Single	6 (31.6%)
Married	12 (63.2%)
Divorced/Widowed	1 (5.2%)
Number of LAP-ST sessions, mean (SD)	5.7(3)
Median	6
Minimum	1
Maximum	12
Depressive episode, and did 1 session only, N	1
Psychotic episode and did 1 session only, N	1
Primary Psychotic disorders (schizophrenia and schizoaffective), N (%)	11 (50%)
Primary Mood Disorders, N (%)	11 (50%)
Depressive Episodes:	
Unipolar depression, N	8
Bipolar depression, N	3

*N=19, (3 missing data)

Table 2. Electric Parameters of first session of LAP-ST, median (IQR) (N= 22).*

Current (mA)	500 (500-500)
Charge (mC)	48 (48-48)
Dynamic Impedance (Ohm)	319.5 (296.5-389.5)
Sodium thiopental (mg)	250 (250-250)
Succinylcholine (mg)	40 (40-40)
Motor seizures (Sec)	31 (19.3-34)
EEG seizures (Sec)	33.5 (24.3-36)

*IQR=Interquartile range

Table 3. Electric Parameters of Subsequent sessions of LAP-ST, median (IQR) (N=22).*

Current (mA)	500 (500-512.5)
Charge (mC)	288 (288-288)
Dynamic Impedance (Ohm)	304.8 (282-321.25)
Sodium thiopental (mg)	250 (250-250)
Succinylcholine (mg)	40 (40-40)
Motor seizures (min) *	24.3 (18.3-31)
EEG (min)	31 (23.8-43.25)

*IQR=Interquartile range

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Chapter 13 General Discussion

GENERAL DISCUSSION

13.1. Summary of key findings

This thesis addresses several questions in the field of epigenetics and brain stimulation in order to provide a step forward in furthering our understanding in each of these areas. Much work needs to be done, but we hope that this work not only gets us closer to advancing these fields, but also sheds light on the important questions in these fields, which will guide the reader to the next steps of inquiry.

Section I, Chapter 2 started with a discussion of the definition of epigenetics and goes on to systematically review and explore the studies in the literature related to the association between epigenetics and depression and/or suicide. Based on pre-specified terms and criteria, sixteen studies met the inclusion criteria. I found that epigenetic changes seem to have associations with both depression and suicide. Several studies showed that hypermethylation of Brain-derived neurotrophic factor (BDNF) is specifically involved in suicide. Tropomyosin receptor kinase B (TrkB) hypermethylation was also shown to be associated with suicide in several studies. Future research is needed in a larger sample to further characterize and replicate these changes.

Chapter 3 explores the impact of trauma and PTSD beyond the affected individual. It not only examines the possibility that psychological trauma can affect the biology of the individual (by review of the current literature), but also that epigenetic alterations related to trauma might be passed on to offspring. Thus, the main aim of this chapter is to review the emerging literature on evidence of transgenerational inheritance via DNA methylation in humans due to trauma exposure. This chapter found an accumulating amount of evidence of an enduring effect of trauma exposure being passed down to offspring transgenerationally via DNA methylation alterations, which has the capacity to (epigenetically) change the expression of genes and the metabolome. This chapter provides an overview of where we stand in this area and a clearer view of where we should go in terms of future research directions.

To overcome an important limitation of the reviewed studies in chapters 2 and 3 (and a common limitation in the field of epigenetics in general), which is that epigenetic studies are commonly underpowered, **Chapter 4** presents a **combined effort by creating a consortium** for detecting epigenetic markers in PTSD. This consortium has led to the publications of combined analysis of over 34 centers. Here, we describe the purpose and methods of this international consortium that was developed to promote collaborative studies on the role of epigenetics in PTSD. We brought together investigators representing seven cohorts (total N=1147) that include detailed information on trauma exposure, PTSD symptomatology, and genome-wide DNA methylation. The objective of this consortium was to increase the analytical sample size by pooling data and combining expertise, in order to identify the DNA methylation patterns associated with PTSD. Several quality control and analytical pipelines were evaluated for their control of genomic inflation and technical artifacts, and a joint analysis procedure was established to derive comparable data over the cohorts for meta-analysis. The proposed ways help with dealing with type I error inflation and discuss the advantages and disadvantages of applying robust error estimates. To evaluate our pipeline, we report results from an Epigenome-Wide Association Study (EWAS) of age, which is a well-characterized phenotype with known

epigenetic associations. Overall, while EWAS are highly complex and subject to similar challenges as Genome-Wide Association Studies (GWAS), we demonstrated that an epigenetic meta-analysis can be well-powered to identify epigenetic associations. This study can be used as a framework for consortium efforts for EWAS.

Epigenetics may also play a role in resilience against mental illness. Moving from the molecular level to the clinical level as it relates to resilience, **Chapter 5** presents a 3-year longitudinal clinical observation study in U.S. military veterans to evaluate the correlation and role of resilience and resilience factors in predicting suicidal ideation and attempts. In this study of 178 veterans, we found that higher resilience at baseline predicted lower suicidality at follow-up, suggesting a protective effect for resilience. Within the specific domains of resilience, secure relationships and positive acceptance of change significantly predicted lower suicidality. The findings indicate the importance of developing programs to boost resilience as part of clinical care or as preventative efforts.

Chapter 6 examines if there are racial differences to the effect of childhood trauma, and how resilience influences the effect of adverse childhood experiences (ACE) on the development of depressive symptoms. This is an area that is much less studied. The specific aims of this study were to examine: 1) whether there is a dose-response relationship between trauma and depressive symptoms; 2) whether early trauma affects European Americans (EA) and African Americans (AA) in a similar fashion; and 3) whether resilience mitigates the effect of trauma.

This was a cross-sectional study of 413 subjects who were 19 years or older. ACEs were significantly associated with depression severity in a dose-response fashion ($p < 0.001$). Notably, AAs had lower depression scores at low to moderate levels of ACEs than EAs but reported comparable levels of depression with severe exposure to ACEs (p interaction=0.05). In both EAs and AAs participants, those with high and medium levels of resilience showed less depressive symptoms when compared to those with low resilience ($p < 0.05$).

Despite the limitation that this was a cross-sectional study, it provided initial evidence to be examined in a longitudinal study that higher resilience mitigated the impact of childhood adversities on depressive symptoms in young adults. In addition, the study points to the racial difference in resilience to ACE, which points to the biological component of resilience as a likely candidate.

In order to examine other non-biological factors that can possibly moderate the effect on either making individuals more vulnerable to developing PTSD or to boost resilience, we performed a cross-sectional study to examine the relationship between moral injury and PTSD, and the impact of religious involvement (RI) on that relationship presented in **Chapter 7**. Religiosity and other moral values are used by some individuals to boost resilience. Traumatic experiences can cause ethical conflicts. Moral injury has been used to describe this emotional/cognitive state and may either contribute to the development of PTSD or block its recovery. This study of 120 veterans found a strong relationship between moral injury and PTSD symptoms ($r=0.54$, $p<0.0001$), and between moral injury and RI ($r=-0.41$, $p<0.0001$), but only a weak relationship was found between RI and PTSD symptoms ($r=-0.17$, $p=0.058$). RI did not mediate or moderate the relationship between moral injury and PTSD symptoms in the overall sample. However, among veterans who were in non-Middle Eastern conflicts, a significant

buffering effect of religiosity was found. The explanation is not clear, but may be due to age differences with different adherences to religious values.

Section II:

While shifting from the epigenetics to the brain stimulation section, **Chapter 8** reviews pertinent studies aimed to avoid relapse and recurrence of depressive symptoms. An acute course of ECT can lead to remission of depressive symptoms in a substantial portion of treatment-resistant patients, but many patients relapse after the ECT course. Thus, this chapter reviews the studies of continuation ECT to examine how to keep patients doing well. The chapter reviews published clinical trials until April 2014. Most studies suggest that combined continuation of ECT and continuation of pharmacotherapy are more effective together than either alone for preventing relapse. Of interest, this work was published prior to the multicenter randomized clinical trial: Prolonged Remission in Depressed Elderly (PRIDE) and has come to the same conclusions as the PRIDE clinical trial. This systematic review found that combined continuation of pharmacotherapy plus ECT is better than either alone.

Although **Chapter 8** provides important insights regarding symptom remission and relapse prevention, symptom remission is not sufficient for individuals' well-being and functioning. Improvement in the well-being and functioning of patients is achieved by improvement in health-related quality of life (HRQOL), which is also important in its own right. Thus, in **Chapter 9**, we analyzed data from the PRIDE study to examine whether ECT plus medications ("STABLE + PHARM" group) had superior HRQOL compared with medications alone ("PHARM" group) as a continuation strategy after successful acute right unilateral ECT for major depressive disorder (MDD). The PHARM group included venlafaxine plus lithium. We hypothesized that scores from the Medical Outcomes Study Short Form-36 (SF-36) would be higher during continuation treatment in the "STABLE + PHARM" group versus the "PHARM" group. Remitters to the acute course of ECT were randomized to 6 months of "STABLE + PHARM" versus "PHARM." One hundred twenty patients ≥ 60 years old were included. The "STABLE + PHARM" received the same medications (venlafaxine and lithium), plus 4 weekly outpatient ECT sessions with additional ECT sessions as needed. "STABLE + PHARM" group had a 7-point advantage (3.5-10.4, 95% CI) for Physical Component Score of SF-36 ($P < 0.0001$), and an 8.2-point advantage (4.2-12.2, 95% CI) for Mental Component Score ($P < 0.0001$). Additional ECT resulted in overall net health benefit. Consideration should be given to administration of additional ECT to prevent relapse during the continuation phase of treatment of MDD.

Although depressive episodes are the most common indication for ECT in the United States (Patel, Sreeram, Thakur, Bachu, & Youssef, 2019), there are several other potential indications that are still under investigation for ECT such as PTSD (Chapter 11) and novel techniques of administration such as LAP-ST (Chapter 12). **Chapter 10**, as its title indicates, provides a charting of "the ECT Course: Where have we been? Where are we headed?" More specifically, it discusses and reviews the current knowledge of ECT in general, while discussing several indications as well as patient selection, choice of electrode placement and stimulus parameters, augmentation with pharmacotherapy, and the use of continuation/maintenance treatment. Then, it discusses future lines of investigations in ECT including research into

biomarkers and neuroplasticity related to ECT response, as well as new, investigational methods of ECT delivery.

Chapter 11 switches gears from some of the well-established indications for ECT to an investigational indication of ECT, namely treatment-resistant PTSD. There is little to offer patients who do not respond to standard interventions of psychotherapy and pharmacotherapy for PTSD. Thus, the objective of this chapter is to systematically review human data on whether ECT is effective in PTSD. The systematic literature review was performed through August 2016 for clinical studies and case reports examining the efficacy of ECT for PTSD symptoms. The literature search generated 3 retrospective studies, 1 prospective uncontrolled clinical trial, and 5 case reports. Given the small sample size and lack of a large randomized trial, it was not clear whether favorable outcomes were attributed to improvement in depression (as opposed to core PTSD symptoms) given that most patients underwent ECT for depression as well. Thus, current efficacy data are inconclusive on the effects of ECT on PTSD symptoms. Randomized controlled trials are necessary to examine the use of ECT in medication-refractory PTSD patients with and without comorbid depression. Subsequent studies may address response in PTSD subtypes and the use of novel techniques such as memory reactivation before ECT.

Chapter 12 focuses on another form of investigational trial in humans. I conceptualized a proof of concept (POC) trial to examine if low current pulse amplitude (500-600mA) used to induce seizures during a full acute course in humans can retain efficacy while reducing cognitive side effects of ECT. The standard clinical current amplitude of 800-900mA may be too high, thus driving the electric field deeper to areas responsible for memory and cognitive side effects such as the hippocampus and temporal lobe. These cognitive side effects of ECT can discourage some patients indicated for ECT from receiving ECT and also affect some others after an acute course of ECT. I have called this technique Low Pulse Amplitude Seizure Therapy (LAP-ST) given that seizures (not convulsions) are the active ingredient needed for therapeutic response (even if the convulsions are blocked by a muscle relaxant). Reducing pulse amplitude has been shown in computer models to improve focality of the electric field, thus mitigating cognitive side effects. Thus, **Chapter 12** examines in a POC open-label trial the feasibility, safety, and cognitive side effects of LAP-ST, if any.

Patients with treatment-resistant primary mood disorders (in depressive episodes), or psychotic disorders who were clinically indicated to undergo ECT were offered to be enrolled. The study consisted of a full acute course of LAP-ST. The primary outcome was feasibility of seizure induction. Clinical outcome measures were: time to reorientation (TRO), Mini Mental State Examination, Montgomery–Åsberg Depression Rating Scale, Brief Psychiatric Rating Scale, and Clinical Global Impression Scale. Twenty-two patients consented for enrollment. We found that LAP-ST is feasible with a quick median Time to Re-Orientation (TRO) of 4.5 minutes (TRO being the best acute predictor of memory and cognitive side effects as shown in several studies). Treatment was efficacious for both depressive and psychotic symptoms. These results, although preliminary, suggest that the LAP-ST technique may result in less cognitive side effects with comparable efficacy when compared to the standard ECT techniques.

13.2. Limitations & Challenges

Some of the important limitations encountered in epigenetics studies are described in the individual chapters. This thesis, (and the epigenetic studies it is based on), also have some of these limitations. One of the most important limitations may be the small to moderate sample size of many studies in epigenetics, limiting the power to detect significant changes. It is very difficult for most centers to gather a sufficiently large sample size given the huge participant numbers as well as the financial resources needed. Thus, it is crucial for the field to collaborate in organized consortia and pool data to generate sufficient power to detect actual differences and to perform replication studies.

Another limitation inherent to epigenetic studies is their interpretability of causality rather than associations. When associations are found, it is still unknown if the found epigenetic dysregulation caused either disease or resilience, whether it is a consequence, or a mere epiphenomenon. Thus, once a replicable association is found, more focused research is needed to unravel these issues. Disentangling these processes will require more longitudinal studies.

A third difficulty that can be a limitation of certain epigenetic studies is the dynamic nature of epigenetic regulation. This can take many forms including the possible observer effect known in quantum mechanics: where the mere act of observing might change the findings, i.e. the experimental procedure itself may result in epigenetic changes. For instance, while observing the behavioral impact of epigenetic alterations in an experiment, behavioral testing in that very experiment in animal models may be stressful enough to induce epigenetic changes (Stankiewicz, Swiergiel, & Lisowski, 2013). It has also been shown that repeated testing can induce epigenetic changes related to learning and memory [2].

Fourthly, limitations in the tools used and cell-type specificity can mislead us. For example, proxy samples are used (like blood or saliva) for brain-mediated disorders. However, cell-type specificity (Davies et al., 2012) may limit the data extracted from proxy sites. Since sampling brain tissue in living individuals cannot be done for this purpose, this makes it a hard dilemma to solve. We hope more correlation studies between brain and peripheral samples can be done (Hannon, Lunnon, Schalkwyk, & Mill, 2015; Smith et al., 2015) to try to get us as close as possible to the needed answer.

Regarding limitation of the brain stimulation studies, they are described in the individual chapters. However, we would like to add here that important limitations in the field relevant to ECT and general brain stimulation studies are that we still lack some important knowledge of the best combination of parameters to use to optimize efficacy and minimize side effects. There is still a need for studies that examine this further. Some of these questions may need to be examined first in animal models as a platform to optimize the parameters. This may apply to ECT as well as other modalities such as transcranial magnetic stimulation and deep brain stimulation in terms of optimizing which combination of parameters to be used in each case.

Another limitation is the lack of enough predictors and precision medicine variables in the field of ECT (and brain stimulation in general). Predictors and precision treatments are important areas for future studies.

13.3. Future perspectives

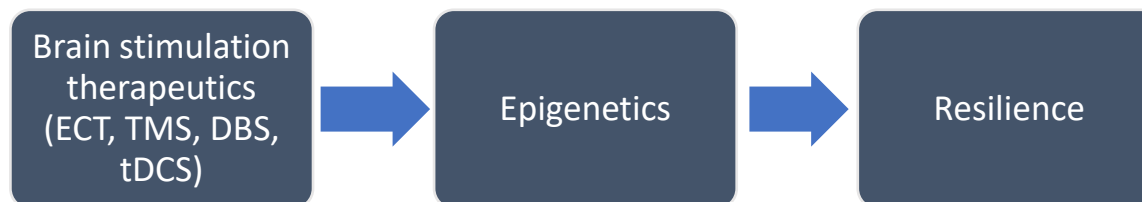
Future perspectives of research would be to address the above limitations and challenges in the field by performing large-scale, concerted longitudinal studies encompassing multi-level variables in the biological, psychological, social and spiritual domains. Given the early phase of studies and the explorative nature of most epigenetic studies, it would also be important to reproduce relevant findings in independent cohorts and across people with different backgrounds.

Some other unique future directions that constitute an overlap and interaction between the field of epigenetics and the field of brain stimulation are also worth concluding and are outlined below.

Next steps after the current work related to the overlap and interaction between the fields of epigenetics and brain stimulation:

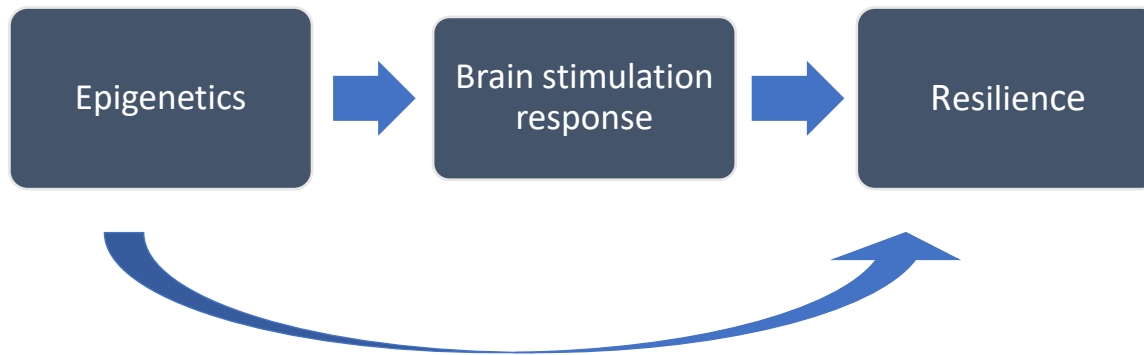
Although the two main sections of this thesis, namely brain stimulation on one side and resilience and epigenetics on the other side, are not always related, some preliminary studies in the literature have examined the relationship and interactions between brain stimulation modalities and epigenetics and between epigenetics and resilience. Although these studies are preliminary, there is enough evidence to suggest some validity in the interactions depicted in **Figures 1 and 2**, suggesting that brain stimulation interventions may lead to epigenetic changes. However, I believe that future research is much needed in these areas. I hope that this thesis has helped answer some of the needed questions and brought our understanding a step forward. In addition, I hypothesize that brain stimulation interventions might improve resilience (some support of this hypothesis comes from preliminary data from our lab not discussed here). These relations might be mediated by epigenetics (see **Figure 1**). These are very important areas to be explored further in the future.

Figure 1: Mediation model.



Mediation model: Epigenetics mediate the relationship between brain stimulation modalities and resilience. Brain stimulation may cause epigenetic alterations, which in turn would boost resilience (Youssef. Brain stimulation, epigenetics and resilience, submitted).

Figure 2: Predictor model



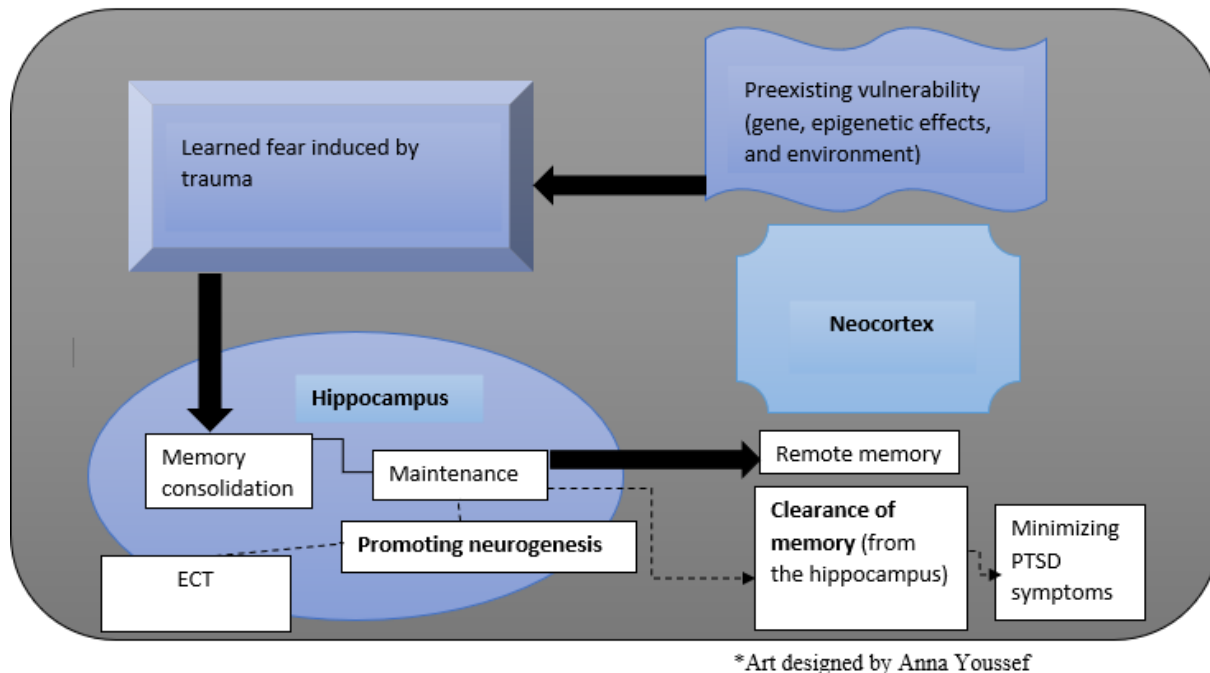
Predictor model: Epigenetics markers may predict treatment response or remission from brain stimulation therapeutics with no direct correlation with resilience. In addition, epigenetics might predict response to brain stimulation intervention and in turn, brain stimulation intervention might improve resilience (Youssef. Brain stimulation, epigenetics and resilience, submitted).

On a related front, epigenetics' signature might provide markers of treatment response for brain stimulation interventions (see **Figure 2**). If this predictor relationship pans out, it could have very important clinical value and implications. Clinically, a simple blood test or saliva swab (if proven to correlate enough with target brain areas) could differentiate the group of patients who would benefit from a course of ECT, for instance, and the group who would not benefit. This also could be an important field that should be further explored in the future.

Another area of future direction, as described in Youssef 2018 is depicted in **Figure 3** (Youssef, 2018). I hypothesized that clearance of traumatic and/or PTSD-related memories might be induced after provoking traumatic memories, then giving an ECT stimulus. This may work, at least in part, via induction of neurogenesis. A possible way this may happen is either via a direct electric stimulation inducing neurogenesis or via an epigenetic mechanism inducing neurogenesis (Youssef, 2018), which may in turn help clear traumatic memories and increase the resilience threshold. One demonstration that may hold relevance to solving this mystery is seen in the results of a recent study published in *Nature* demonstrating that hippocampal adult neurogenesis confers resilience towards stress (Anacker et al., 2018).

As indicated elsewhere, traumatic memories “generate some of the most persistent memories and are the hardest to erase. This might be explained by the cellular mechanism for encoding durable memories” (Youssef, 2018) possibly mediated via epigenetic alterations (Hao, Youssef, Davis, & Su, 2018).

Figure 3. Proposed mechanism for clearance of traumatic memories and neurogenesis.



Adopted from Youssef NA. Treating Recalcitrant PTSD with ECT: Are We There Yet? *Psychiatric Times* August 2018 (http://www.psychiatrictimes.com/sites/default/files/legacy/mm/digital/media/psy0818_ezine.pdf)

I hope the body of work in this thesis spurs more research on the relationship between epigenetics and brain stimulation in different laboratories around the world. Moreover, my next steps in my translational research are to investigate these relationships given my interest, prior work, and experience in these areas.

Among several areas, two concrete and immediate areas of future directions of research after my PhD that I plan to investigate (based on my work in the areas of brain stimulation and epigenetics) are as follows:

1. How ECT may improve resilience? Also, if our initial pilot data are confirmed in a larger study, can epigenetics be the (or one of the) mechanistic explanation(s)?
2. Investigate epigenetics' signature or markers of treatment response for ECT. This has important clinical implications, and will help us toward individualized health care.

This of course would be a rich area for collaboration with colleagues and with experts from both the field of epigenetics and that of ECT/brain stimulation. It would also be welcomed for other labs to simultaneously explore this over the next several years, as these questions can have an important impact on the field and on patients' lives.

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Affective disorders, including major depressive disorder (MDD), are a leading cause of disability worldwide with a high burden of morbidity and mortality (World Health Organization, 2017). Affective disorders are also a major risk factor for mortality, including both death due to suicide and other medical conditions.

Affective disorders and depressive episodes have long been recognized to result from an interplay between environmental and biological factors (Wankerl et al., 2014). Epigenetics involves functional modifications of genes that are affected by environmental factors. Thus, epigenetics is likely an important mechanism that contributes to the development of and/or relapse from affective disorders. Several studies showed that certain epigenetic changes (mainly hypermethylation) to be associated with suicide and depression.

Treatment-resistant depression (TRD) is defined as MDD that is not responsive to treatment despite two different antidepressant regimens of adequate duration and dosage; 1 out of 3 patients suffering from MDD has TRD (European Medicines Agency, 2013; Kular & Kular, 2018; U.S. Department of Health and Human Services, 2018). Many patients who do not respond to medications and psychotherapy may have a robust response and remission to Electroconvulsive Therapy (ECT).

Another stress related disorder is Posttraumatic Stress Disorder (PTSD), which also causes substantial burden and suffering (American Psychiatric Association, 2013; Department of Veterans Affairs and Department of Defense, 2017). PTSD has a high prevalence in many countries, and is more prevalent in combat veterans representing up to 17% (American Psychiatric Association, 2013; Department of Veterans Affairs and Department of Defense, 2017; Kessler, Chiu, Demler, Merikangas, & Walters, 2005; Miao, Chen, Wei, Tao, & Lu, 2018). PTSD has a strong genetic component, evidenced by numerous research studies. For instance, twin studies found estimated heritability ranging from 30% to as high as 72% (Sartor et al., 2011; Stein, Jang, Taylor, Vernon, & Livesley, 2002). Transgenerational studies done with children of holocaust survivors show that PTSD is more likely to occur in certain families, for review see e.g. Youssef et al. (Youssef, Lockwood, Su, Hao, & Rutten, 2018). However, PTSD etiologically involves multifactorial processes and a dynamic and complex interplay of different biological systems and psychological trauma (Ratanatharathorn et al., 2017). Even though it is known that PTSD is heritable, the complex underlying genetic architecture of this disorder is unknown (Ratanatharathorn et al., 2017).

Within the nature-nurture discussions, scholars have proposed to study the complex interaction between genetics and the environment in stress-related mental disorders (depression and PTSD) using epigenetics. Similar research has proven fruitful in the field of cancer genomics. This approach may in fact be uniquely suited to be applied to PTSD research, since the effect of trauma on mental health is suspected to be mediated through epigenetic changes. Consequently, there is growing appreciation of the role of epigenetics in the etiology of PTSD.

Potential applications of the knowledge gained for future research of stress related disorders.

- A. Progress in the scientific areas of research of epigenetics and resilience can advance our understanding of the mechanism underlying these stress-related mental disorders and on the other hand may innovate treatment modalities to manage these disorders. The research progress may thereby assist in several manners in the following 2 ways:
 - 1) Sub-classifying PTSD and depressive episodes of mood disorders with the help of epigenetic biological markers. This can improve possibilities in personalizing diagnosis and treatment interventions (Feng & Youssef, 2019).
 - 2) Epigenetics plays an important role in improving our understanding of the interplay between environment and underlying genetics, and identifying mediating biological processes, which may be targeted for modulation by interventions, such as medication, lifestyle and dietary components.
- B. Also, based on the findings of this thesis, the identification of factors and mechanisms involved in co-determining and/or mediating differential susceptibility and resilience to the effects of trauma and other exposures may enable novel preventative and treatment modalities for stress-related disorders. Moreover, brain stimulation interventions also can play important roles in the treatment of stress disorders especially the most powerful of these interventions, namely ECT (Lisanby, 2007; Rush et al., 2006).

Potential applications of the knowledge gained for improving patient care and translational research to prevent and/or treat stress-related disorders.*

Some studies have already identified epigenetics markers that can be tested clinically and can be used in clinical care not too far in the future. This can, for instance, help in attempts for predicting inter-individual differences in response to treatment modalities, i.e. personalized medicine.

Precision medicine can benefit both the patient and clinician in terms of saving suffering, money, time, and frustration (Feng & Youssef, 2019). Epigenetic biomarkers have been suggested (at least in some initial studies) to predict response to pharmacological treatment in depression (Goud Alladi, Etain, Bellivier, & Marie-Claire, 2018). Promising studies found that hypomethylation of genes related to the serotonin transporter (Domschke et al., 2014) and receptor (Wang et al., 2018) predicted impaired response to escitalopram. Also, another study found hypermethylation at a different gene, the IL-11 gene promoter, predicted favorable response to escitalopram and decreased response to nortriptyline (Powell et al., 2013). However, in order to reduce false-positive findings, the field warrants adequately powered studies with an agnostic, genome-wide approach and using a discovery as well as a replication approach.

Regarding epigenetics and ECT, data have both suggested epigenetic modifications by ECT in animals, and on a different front, initial human data examined epigenetics as biomarkers of treatment response (Feng & Youssef, 2019). Initial data of epigenetic modifications produced by electroconvulsive seizures (ECS) has been presented by a review paper (de Jong et al., 2014). This paper reviewed all rodent studies. All studies in the review have multiple epigenetic effects that are suggested to modulate the therapeutic effects of ECT. This included increased histone acetylation in c-Fos, BDNF, and CREB genes, demethylation of the BDNF promoter, and changes in levels of various micro-RNAs.

Initial human studies also examined epigenetics as predictors of treatment response for ECT (Kleimann et al., 2015), and was further supported by a follow-up study (Neyazi et al., 2018). The initial human study found that both ECT remitters and responders had significantly lower baseline methylation percentage in the BDNF promoter when compared to non-responders (Kleimann et al., 2015). They proposed the potential application of BDNF promoter methylation as a predictive biomarker for ECT. The follow-up study examined the methylation of the promoter for p11, (a molecule involved in serotonin signaling and upregulation of BDNF) (Neyazi et al., 2018). The researcher studied p11 promoter methylation in a mouse model of depression, and then did a translational proof-of-concept study in humans.

In mice, ECS responders showed hypermethylation of p11 promoter and increased p11 mRNA expression in the PFC. Patients undergoing ECT showed similar findings in serum samples at all-time points, and patients with higher baseline p11 promoter methylation had significantly less depression after ECT treatment. The results suggest that p11 methylation can predict ECT response, which was tested (Feng & Youssef, 2019).

In a separate patient sample, p11 promoter methylation above 72.15% predicted response to ECT with a positive predictive value (PPV) of 90%. The test identified responders with a sensitivity of 70% and specificity of 73%.

This line of inquiry seems promising, and if pursued, can lead to fruitful epigenetic biomarkers of treatment response and precision psychiatry (Yao et al., 2019).

Further studies should also replicate the information in this thesis regarding that hypermethylation of brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B (TrkB) hypermethylation are potential biomarkers for suicide. This could, if replicated, be used as a biomarker to help in focused efforts of suicide prevention.

Similarly, further replication studies should examine the accumulating evidence suggesting enduring effects of trauma exposure being passed down to offspring transgenerationally via DNA methylation alterations. This can inform and direct further public health efforts in preventing traumatic child abuse and partner violence and boosting resilience among other programs and initiatives.

Policy, stakeholders, and society:

As mentioned above, epigenetic research can provide both mechanistic understanding and biomarkers of treatment response. Policy makers should improve and allocate funding for the development of epigenetic research and support epigenetic consortia to increase sample size and for the statistical power needed for such research, which involves many multiple comparisons and thousands to millions of variables. We have demonstrated such an example in our collective efforts in developing Epigenome-Wide Consortium in PTSD. We demonstrated that epigenetic meta-analysis (as part of combined effort by the consortium) can be well-powered to identify epigenetic associations.

Also, further programs to boost resilience in both civilians and military veterans can be of help as adjunct treatment of depression and PTSD, or in boosting resilience for prior known traumatic events, for example, prior to deployment in order to prevent PTSD in veterans going to combat zones.

Traumatic experiences violating a certain moral code or specific religious beliefs can be associated with moral injury, and possibly block recovery from PTSD and/or depression. Further

allocation of resources to improve future research in areas related to resilience, the relationship between moral injury and PTSD, as well as the impact of religious involvement and other violations of moral values are warranted, as these help in fostering resilience and providing meaning to life.

Another important movement related to policy makers and society at large is improvement of education about the potential benefit of approved brain stimulation modalities such as Transcranial Magnetic Stimulation (TMS) and ECT. Although ECT is the most effective treatment in psychiatry (especially for treatment resistant mood disorder), it has bad publicity due to lack of education of the public, and erroneous media depictions of ECT as a method of torture rather than treatment. This can have deleterious effects on our patients and provide a serious barrier for care. In many cases, this results in avoiding a much-needed lifesaving treatment. Education campaigns about the role of ECT and these misconceptions, in addition to providing resources for studies to improve the cognitive side effects of ECT, would prove very helpful. Another application for this thesis is the use of both medications and ECT, rather than either alone, for maintenance of treatment-resistant patients with depression. We have shown in both a review of the literature, as well as in the analysis of the Prolonged Remission in Depressed Elderly (PRIDE) study, that the combination of ECT with medications helps patients to stay well in remission and also to improve quality of life and overall net health benefits compared with medications alone in treatment-resistant depression.

In order to mitigate the cognitive side effects of ECT that can discourage many patients indicated for ECT from receiving it, we presented a novel and promising technique of ECT named Low Pulse Amplitude Seizure Therapy (LAP-ST). We have presented a proof of concept clinical trial showing it to be feasible, safe, and with minimal or no cognitive side effects compared to the standard ECT techniques. We followed also with another pilot randomized clinical trial (not included in this thesis) using low amplitude-titrated seizure therapy, which was similarly promising (Youssef et al., 2019). A replication with a larger clinical trial may confirm these results can have substantial benefits on patients in terms of minimizing or avoiding memory and cognitive side effects during such treatments.

Much work needs to be done, but we hope that this work does not only get us a bit closer to advancing these fields, but also sheds some light on the important questions in these fields with widespread implication in helping patients and the society at large.

Dissemination towards implementation:

All the studies presented in this thesis have been submitted and are now published in peer-reviewed journals to help dissemination and implementation by clinicians, educators and policy makers. In addition, many of these studies have been presented as abstracts, posters and/or oral presentations in targeted scientific meetings for researchers, and others for clinicians. Some were presented as oral presentations to patient advocacy groups. Some studies also motivated media requests for interviews, and the information was disseminated by the interviews with journalists and presented in newspaper articles about the research and its findings, as well as radio and TV interviews locally, nationally, and internationally in an effort to speed the knowledge dissemination and subsequent implementation (including directions on how it can be implemented). These efforts would benefit the largest number of patients possible, help direct policy, and help decrease stigma of mental illness and of certain treatment modalities.

*Parts adopted from Feng T, Youssef NA. Can epigenetic biomarkers lead us to precision medicine in predicting treatment response and remission for patients being considered for ECT? *Psychiatry Res.* 2019 Oct 28;112659. doi: 10.1016/j.psychres.2019.112659. [Epub ahead of print] PubMed PMID: 31703983.

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Summary

Psychological adaptation or resilience reflects functional, cognitive and/or behavioral adjustment to contextual (environmental) changes that comprises a key factor in psychological well-being. Lack of (or dysfunctional) adaption/resilience might contribute to stress-related disorders such as mood disorders and Posttraumatic Stress Disorder (PTSD). Stress-related disorders are also good models to study the interaction between nature and nurture (as both have significant genetic-environmental (GxE) interaction). They can also help study undesirable changes in neuronal function, adaptability, and neuroplasticity to understand how to reverse them to produce remission of symptoms. At the molecular biological level, epigenetic mechanisms may have an important role in the response to rapid environmental change and adaptation.

This thesis focuses and converges on two promising and rapidly evolving areas of neuroscience and psychiatry. These areas are epigenetics and brain stimulation. These 2 evolving fields can help to modify neuronal function, adaptability, and neuroplasticity and to investigate underlying (biological) processes. Stimulation of the brain also reflects exogenously induced interventions and can be seen as an environmental exposure. Thus, the research compiled in this thesis can be divided in two main sections: 'Epigenetics and Resilience' and 'Brain Stimulation' with ECT as the prototype for the purpose of this thesis.

The first section of this thesis focuses on epigenetics as one possible molecular mechanism underlying risk and resilience to stress-related disorders. It starts by providing an overview of recent advances in epigenetics as biomarkers in depression and an overview on epigenetics and PTSD, especially the evolving transgenerational transmission of the effects of PTSD via epigenetic methylation. It then presents our studies showing that higher resilience (that also have an epigenetic/biological component) predicts lower suicidality at 3-year follow-up, as well as lower depression as shown in another study we conducted. It also examines racial differences on the effect of trauma on development of depression. In a third study, non-biological factors such as moral injury and spirituality were also examined in how it impacts and correlates with PTSD symptoms. Finally, it presents our group efforts of establishing epigenetic consortium in PTSD and pooling data to increase the sample size for well-powered more definitive studies.

The second section of this thesis focuses on electroconvulsive therapy (ECT). It starts by reviewing the best way to prevent relapse after induction of remission (using a clinically-indicated acute course of ECT) for depressive episode of mood disorders. It also reviews other indications for ECT, including the emerging evidence that ECT might be of help in treatment-resistant PTSD, and the future direction for ECT research. As a related inquiry to the common indication of ECT in treatment-resistant depression, it also presents our randomized multicenter clinical trial on the effects of continuation of ECT plus medications versus continuation of medication alone on how the combination has significantly better improvement on quality of life. Then, it discusses cutting-edge research in the field, including research into biomarkers and neuroplasticity related to ECT response.

Moreover, in order to find ways to avoid (or minimize) the cognitive side effects of ECT, we examine a novel and focal form of stimulation: Low Pulse Amplitude Seizure Therapy (LAP-ST) for treatment of depressive episodes. In this clinical trial, we specifically examine the feasibility and preliminary evidence that it has less cognitive adverse effects compared to standard ECT. Our proof of concept clinical trial showed that this technique is feasible, and initial data showed LAP-ST to be safe, and with minimal cognitive side effects compared to the standard ECT while retaining efficacy.

Much work needs to be done, but we hope that the work in this thesis gets us a few steps closer to advancing the fields of epigenetics and ECT. We also hope there will be more examination of the interaction of these fields as presented in the discussion in the last chapter of this thesis, where we present a plan for future steps of research in this direction. We then present an addendum chapter on valorization of this knowledge so as to benefit the society, stakeholders, policy makers, as well as, researchers and clinicians and the efforts made to disseminate this information.

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About the Author

Nagy Youssef's medical training included medical school at Cairo University, Egypt; psychiatry residency at University of South Alabama (Mobile, AL, USA) and postgraduate Consultation-Liaison training at Yale University (New Haven, CT, USA). He also completed a 3-year research fellowship at both Durham VA and Duke University (Durham, NC, USA) and electroconvulsive therapy (ECT) & Transcranial Magnetic Stimulation (TMS) fellowship at Duke University. As a faculty at Duke University, he established and directed a Treatment-Resistant Mood Disorders Outpatient Clinic, supervised residents and medical students in the clinic, in the Duke ECT service, and during weekly ECT rounding. He was one of the ECT and TMS faculty and investigators in the Division of Brain Stimulation & Neurophysiology at Duke. He also taught physicians in the Duke ECT fellowship program and TMS fellowship.

After moving to Augusta, GA, USA, and as a faculty at Medical College of Georgia (MCG), he served as the medical director of acute psychiatric services (psychiatry inpatient and the psychiatric emergency department) at the affiliated Veterans Affairs Medical Center, then developed and currently directs a Mood and Trauma Clinic at MCG, where he also supervises residents and students in the clinic and is an ECT and brain stimulation faculty as well as directs a student clerkship in Mood Disorders.

His primary research interests include mechanistic and therapeutic innovation for treatment-resistant mood disorders, PTSD, and suicide prevention. His methodology and interventions within these areas include: 1) multiple brain stimulation mechanistic probes and therapeutics, including ECT, Transcranial Magnetic Stimulation (TMS), Magnetic Seizure Therapy (MST), and Transcranial Direct Current Stimulation (tDCS), and 2) genomics research. His studies involve primary clinical research, but he has occasionally studied non-human primates and translated the findings to humans.

He has presented his research in over 150 scientific papers and published abstracts. Dr. Youssef has lectured nationally and internationally on many research findings. The research studies he was involved in has been supported by the Department of Veterans Affairs, National Institute of Health, several research foundations, academic institutions, and pharmaceutical and device industries.

His service to the scientific community locally and internationally includes being a member of the Research Ethics Committee for 5 years at the Medical College of Georgia and as a member on the editorial board of several scientific journals.